Ureteric Perturbation and Obstruction

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Abstract

The ureter is a complex organ with unique characteristics, and it responds to the continuous changes in its internal and external environment. The initiation and propagation of ureteric peristalsis along the different ureteric segments and its adaptability to various flow conditions are not clearly understood. The hypothesis of this thesis postulates that the ureter can adapt to various physiological and pathological conditions due to the difference in the contractile and pharmacological properties of its segments.

To investigate this hypothesis; the ureter was divided into five segments, and the basal contractile properties of these segments were investigated and compared.

The effect of cholinergic pharmacological manipulation on the ureter was investigated in the upper, middle and lower ureteric segments. Also, the effect of ureteric obstruction caused by kidney stones was investigated in the upper and lower ureteric segments.

Methods:

The investigation of ureteric basal and pharmacologically modulated contractility and obstruction by stones were conducted in organ bath experiments using ureteric segments from freshly slaughtered pigs.

Results & Conclusion:

The different ureteric segments demonstrated variability in their contractile behaviour. The ureter demonstrated a spasmodic response to obstruction caused by the stone passage. The difference in ureteric segment contractility and response to pharmacological manipulation has provided further insight into the complexity and adaptability of the ureter to various conditions and further targetted investigations to explore a new approach to medical expulsive therapy to facilitate the passage of ureteric stones.

Dedication

Grouse Mountain, Vancouver.

I am not a mountain climber; I saw the mountain as I arrived at the waterfront; I thought to myself, I would like to climb this mountain. I walked for many hours, and I was there at the foot of the mountain. I was not prepared or ready to climb, but I just wanted to. Few hours later, I was at the top.

This thesis resembles my mountain climbing experience; I was not prepared or ready, but I started it a few years ago. The path was difficult, treacherous and sometimes painful but it's here now, a reality.

Even when not ready, dream, take the chance, keep going, learn and grow.

I dedicate the effort of this thesis to my father Mohamed, my mother Moshira, my wife Amina, my son Adham, my brother Mohannad, my sister May.
Thank you all for lighting my life and for your love and support.

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I would also like to thank all my supervisors, friends and colleagues for their insights, contributions and support.

Author's declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's *Regulations and Code of Practice for Research Degree Programmes* and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

SIGNED: ..... DATE:.....

List of Publications and abstracts

1- D. Carugo, M. ElMahdy, X. Zhao, M.J. Drake, X. Zhang, F. Clavica. An artificial model for studying the flow dynamics in the obstructed and stented ureter. EMBC conference – Osaka Japan 2012.

2- Carugo, D., Elmahdy, M., Zhao, X., Drake, M. J., Zhang, X. and Clavica, F. (2013b) 'An artificial model for studying fluid dynamics in the obstructed and stented ureter', in *Proceedings of the Annual International Conference of the IEEE Engineering in Medicine and Biology Society, EMBS*. doi: 10.1109/EMBC.2013.6610754.

3- Clavica, F., Zhao, X., ElMahdy, M., Drake, M. J., Zhang, X. and Carugo, D. (2014) 'Investigating the flow dynamics in the obstructed and stented ureter by means of a biomimetic artificial model', *PLoS ONE*, 9(2).

4- Elmahdy, M., Drake, M. J. and Vahabi, B. 'Comparison of the basal phasic activity of the pelvicalyceal system , renal pelvis , upper , middle and lower segments of pig ureter' International Union of Physiological Sciences – Birmingham 2013.

5- Elmahdy, M., Drake, M. J. and Vahabi, B. Investigation of the basal and cholinergic phasic activity of upper versus lower segments of the pig ureter. University of the West of England - Centre for research in Biosciences annual meeting 2013.

6- Elmahdy, M., Vahabi, B, Drake, M. J. Comparison of the basal and modulated phasic activity of the pelvicalyceal system, renal pelvis, upper, middle and lower ureteric segments. Royal society of Medicine - oral presentation 2014: Prize: Second place

7- Elmahdy, M., Vahabi, B, Drake, M. J. Comparison of the basal activity of the pelvicalyceal system, renal pelvis, upper, middle and lower ureteric segments. Bristol Urological Institute 21st annual scientific meeting 2014. Best scientific poster and oral presentation.

8- Investigation of the basal spontaneous activity, the effect of Cholinergic receptor modulators and obstruction on the pig upper Urinary tract. International continence society – Gothenburg Sweden 2019.

Contents

Figures and tables

Chapter 1

Introduction & overview

1.1 Introduction

Ureteric peristalsis represents a complex physiological phenomenon and exhibits some unique properties and some that are shared with other organs. The ureter shares a few pharmacological and anatomical similarities with the Fallopian tube and vas deferens, respectively. The fallopian tube shows rhythmic contractions and is responsive to prostaglandins as the ureter and the vas deferens resembles the ureter in its uniformity of structure over its length (Boyarsky & Labay, 1971). No other organ in the body, except the urethra, transports urine. The ureter has the ability to alter readily dimensions of the lumen in response to the amount of urine produced by the kidney and in pathological conditions. It propels small boluses of urine during dehydration but can dilate and still enable a wide column of fluid to flow in diuresis (Boyarsky & Labay 1971). Also, in conditions where there is an intrinsic (e.g. stone, tumour) or extrinsic (e.g. retroperitoneal fibrosis, pregnancy) cause of obstruction, the ureter can respond to these challenges and maintain a flow of urine, that will preserve renal function, unless the obstruction is severe.

To develop a conceptual understanding of ureteric dynamics it is necessary to consider the ureter as more than just a simple conduit that contracts repetitively to maintain peristalsis. Overall function is integrated by the presence of typical smooth muscle cells, atypical pacemaker cells and coordination by the autonomic nervous system (Constantinou & Djurhuus, 1981). A better understanding of how the ureter functions under different physiological and pathological constraints is therefore essential. A focus of research has been

to understand how the ureter functions in pathological conditions such as obstructive stone disease to better understand how it may expel renal stones. However, less research has been spent on how the ureter may adapt to maintain near-normal function under a range of pathological constraints and has been the focus of the work in this thesis.

1.2 Ureteric anatomy

The ureter is a hollow organ which arises from the collecting system of the kidney and terminates at the bladder. In humans and pigs the kidney is multicalyceal and minor calyces combine to form several major calyces that open into the renal pelvis. In small mammals such as the rat, guinea pig and the rabbit, the kidney is unicalyceal. A detailed understanding of the renal anatomy further elucidates the origin of the collecting system. The kidney structure is divided into the cortex and medulla; the functioning unit of the kidney is the nephron. medulla is subdivided into eight to eighteen striated well-defined, conically shaped regions that is referred to as renal pyramids. These are separated by sections of renal cortex called *columns of Bertin.* Each renal pyramid terminates centrally in a papilla.

Microscopically, the renal collecting system originates in the renal cortex at the glomerulus as filtrate enters Bowman's capsule. Collectively the glomerular capillary network and Bowman's capsule form the renal corpuscle. The filtrate is initially collected in Bowman's capsule and then moves to the proximal convoluted tubule. The proximal tubule is located more profound into the cortical tissue where it becomes the loop of Henle. Within the renal medulla, the loop of Henle reverses course and moves back toward the periphery of the kidney, becoming the distal convoluted tubule; which ultimately returns to a position adjacent to the originating glomerulus and proximal convoluted tubule. The distal convoluted tubule turns once again towards the interior of the kidney, becoming a collecting tubule.

Collecting tubules from multiple nephrons join into a collecting duct that reaches inward through the renal medulla and ultimately empties into the apex of the medullary pyramid; the renal papilla, which is the first gross structure of the renal collecting system. Usually there are seven to nine papillae per kidney, but this number is variable and can range from four to eighteen. Each papilla is cupped by a minor calyx. Groups of minor calyces join to form a major calyx and the major calyces combine to form the renal pelvis (Elkoushy & Andonian, 2015).

The ureters are situated in the retroperitoneum, lateral to the tips of the transverse processes of the lumbar vertebrae and extends down to the bladder crossing the abdominal and pelvic structures .

In the abdomen; the ureter originates at the level of the renal artery and vein and is located posteriorly. The pelviureteric junction usually corresponds with the second lumbar vertebra on the left side, and the right is situated marginally lower.

The course of the ureter continues anteriorly over the psoas major muscle, traversing inferior to the gonadal vein at the level of the lower pole of the kidney. The ureters subsequently course medial to the sacroiliac joint and then course laterally in the pelvis. The colon and its mesentery are located anteriorly to the ureters. Precisely, the cecum, appendix, and ascending colon are situated over the right ureter; the descending and sigmoid colon lie over the left ureter.

As the ureter enters the pelvis, it crosses anterior to the iliac vessels, usually at the bifurcation of the common iliac artery. At this level, the ureters are within 5 cm of one another before they separate laterally. The ovarian vessels are located in the suspensory ligament of the ovary; the infundibulopelvic ligament and traverse anteriorly to the ureter and lateral to the iliac vessels. The ureters then course lateral to the ischial spines before coursing medially to enter the bladder at the base. The anteromedial aspect of the ureter is covered by

peritoneum, and the vas deferens runs anteriorly to it. The ureter proceeds with the inferior vesical neurovascular pedicle into the bladder. In females, the ureter continues posterior to the ovary and then under the broad ligament and within the cardinal ligament as the uterine artery crosses anteriorly in the rectouterine fold of peritoneum.

The international anatomical terminology has divided the ureter into an abdominal segment, pelvic segment and an intramural segment. Also, a different nomenclature of the ureter has been described based on its anatomic relationship to the surrounding structures. The ureter is divided into upper, middle, and lower segments. The upper ureter extends from the renal pelvis to the upper border of the sacrum. The middle ureter continues from the upper to lower borders of the sacrum. The distal ureter continues from the lower border of the sacrum to the bladder (Lescay and Tuma, 2019; Soriano and Leslie, 2019).

In humans the length of the ureter in adults is between 25–30 cm and in new-born infants it is between 6.5–7.0 cm long (Anderson & Kabalin 2007). The diameter of the ureter is between 1.5–6 mm; this will depend on the segment of the ureter; other studies show that the average diameter of the ureter is between 2-4mm in humans (Zelenko *et al.*, 2004). The renal pelvis is funnel shaped and is a wider diameter ranging between 7mm to 10mm in adults (Emamian *et al.*, 1993). In the upper ureter the diameter is larger than the middle and lower ureteric segments. The study by Carugo *et al.*, (2013) demonstrated the size difference between the ureteric segments in the pig model; the upper ureter mean diameter of 6.0±1.3mm and tapers down to 2.4±0.6mm in the middle ureter and the lower ureter 2.2±0.6mm; these figures represent the average diameter of each segment in that study.

As the ureter enters the bladder, it runs obliquely through the bladder wall, and is enveloped by the muscular layer of Waldeyer. Here the ureter merges with bundles of detrusor muscle

of the bladder wall to form thicker longitudinally arranged muscle bundles. This oblique entry through the bladder wall forms an anti-reflux mechanism that prevents retrograde flow of urine from the bladder back into the ureter. This intramural segment of the ureter is about 0.5 – 0.8 cm in neonates and 1.2 to 2.5 cm in adults (Fröber, 2007).

The blood supply to the ureter originates from the renal arteries, the testicular/ ovarian arteries, but also direct branches from the aorta and common and internal iliac arteries. Branches of the renal artery supply the renal pelvis and the upper ureter. The middle part of the ureter is supplied by branches from the testicular or ovarian artery. The aorta and the common iliac arteries play a role in supplying vascular branches to the ureter.

The pelvic or distal segment of the ureter is supplied mainly by branches of the internal iliac artery. It is important to recognise the direction of the nutrient blood supply of the ureter as this may affect the surgical approach; hence, not to compromise the blood supply to a particular segment. These nutrient arteries tend to approach the ureteric wall from one direction. In the upper segment of the ureter, i.e. above the pelvic brim, these arteries come from a medial direction. Below the pelvic brim, the ureteric segment is supplied by branches that come from the lateral aspect. The distal segment of the ureter is occasionally supplied by the middle rectal artery. In males, the internal iliac artery or the umbilical artery may give rise to the artery of the vas deferens which gives a branch to the lower segment of the ureter. In females, the distal part of the ureter is supplied by the uterine artery (Fröber, 2007).

The anatomical division of the ureteric segments implies that there is a functional difference between the segments, but there is no clear evidence from the literature about possible functional variability between the three segments has been investigated.

1.3 Innervation of the ureters

Ureteric innervation originates from both divisions of the autonomic nervous system. The thoracolumbar innervation from T10-L1 provides sympathetic innervation via the renal plexus and ganglia. The renal and upper ureteric branches from the inter-mesenteric plexus proximally and the middle ureteric branch of the inter-mesenteric plexus in the middle segment of the ureter. In the pelvis, the ureter receives parasympathetic innervation from the pelvic splanchnic nerves and the inferior hypogastric plexus.

The ureter smooth muscle cells are a syncytial type of without discrete neuromuscular junctions (Burnstock, 1970). Ureteric peristalsis may persist after transplantation (O'Conor & Dawson-Edwards, 1959) or denervation (Wharton, 1932), as the spontaneous ureteric activity may occur in isolated in-vitro segments (Finberg and Peart, 1970). The evidence from the literature denotes that ureteric peristalsis can occur without innervation, although, the nervous system provides a modulating role in ureteric peristalsis. Morita et al. (1987) describe the autonomic nervous system may affect urine transport by altering both the frequency of peristalsis and the volume of the urine bolus.

1.4 Ureteric Histology

The ureter is composed of mainly three layers, the adventitia, the muscle layer and the mucosa.

1.4.1 Adventitia

The adventitia completely envelops the whole ureter. It is composed of areolar and fibro-elastic connective tissue with variable thickness. The ureteric blood supply runs longitudinally under this layer. It provides a cover for the ureter which allows it to slide freely and change

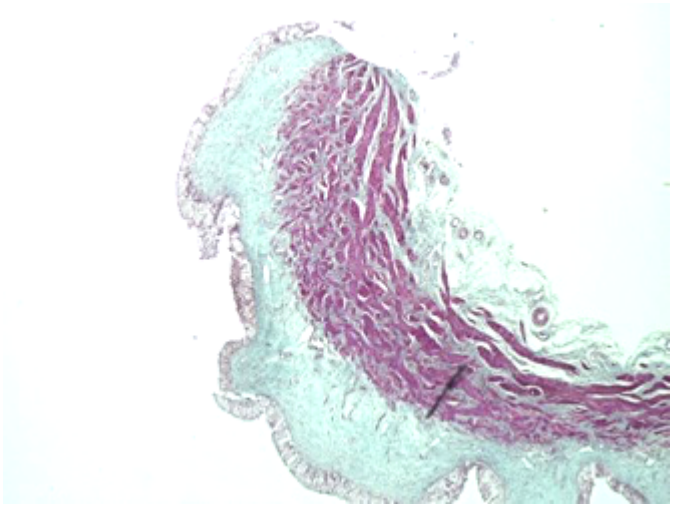
its calibre during peristalsis. The adventitia starting at the renal pelvis is continuous with the pelvic fascial sheath. The structure of the adventitia is altered at the distal end of the ureter as it becomes thicker and more prominent; this fibromuscular layer forms a cover around the terminal 3 to 5 cm of the ureter - Waldeyer's sheath described in 1892 (Scheuerlein et al. 2017). The space between the ureteric wall and Waldeyer's sheath contains loose connective tissue that aid the movement of the ureter into the bladder during peristalsis. This sheath has an important functional role for the lower ureteric segment and the vesicoureteric junction as it serves as part of the anti-reflux mechanism described by Wood Jones 1953 (Radmayr *et al.*, 2009). It also forms a connection between the mesodermal-derived ureter and the detrusor muscle, derived from endoderm.

1.4.2 Muscle layer

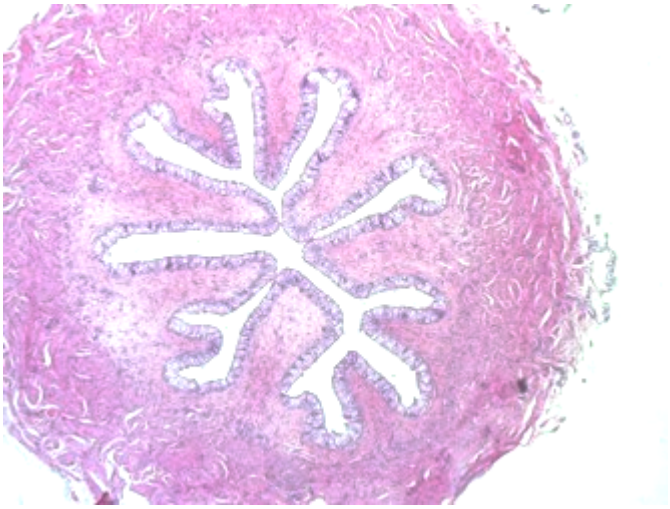
The ureter is a muscular tube which starts at the renal pelvis; the upper third has a thin muscular layer that consists of smooth muscle fibres that are arranged in small bundles and separated by connective tissue. The pelviureteric junction has a gradual change in calibre from the renal pelvis to the upper part of the ureter. During embryonic development, the cranial end of the ureteric bud grows to form the renal pelvis, and the rest of the tube continues to lengthen caudally. In the early developmental stages, a mesenchymal cell arrangement has a circular orientation around the ureter, and as it extends, the muscle fibres develop a more longitudinal orientation. The growth of the ureter in both directions results in a mixed orientation of fibres throughout the collecting system; with a preponderance of either circular or longitudinal fibres at different locations along the ureter: the renal pelvis (Fig 1) tends to have more circular oriented fibres because its growth was along a transverse

axis. The pelviureteric junction and upper ureter (Fig.2) has more circular fibres than longitudinal and as the ureter grows longitudinally, it has an equal distribution of fibre orientation (Spronck *et al.*, 2014).

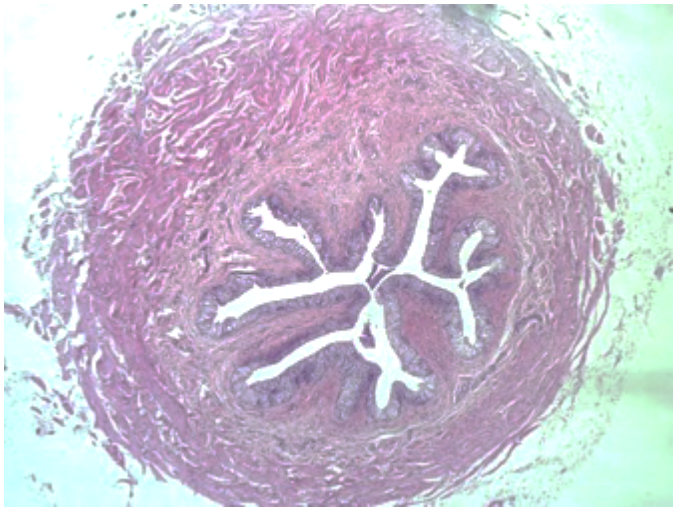
The lower ureter (Fig.3) has longitudinal fibres and some smaller circular bundles. This segment has a longitudinal orientation of fibres as it approaches the ureteric orifice where the ureter continues into the trigone.



Chapter 1 – Fig 1. Renal Pelvis (Mag:4x0)



Chapter 1 – Fig 2. Upper ureter (Mag:4x0)



Chapter 1 – Fig 3. Lower ureter (Mag:4x0)

1.4.3 Mucosa

The mucosa of the ureter is composed of transitional epithelium. The ureteric urothelium lies over the lamina propria, which itself is composed of loose tissue and fibrous bands that attach the basal epithelial cells to the muscle layer. As this sub-urothelial layer is not rigid and loosely attached, in the resting state it forms longitudinal folds that can distend easily and stretch the urothelium to allow for a potential space that that may undergo gross structural changes of the ureter during peristalsis. The transitional epithelium also provides a barrier against urine diffusion into the interstitial space and will layer serves as a protection against the acidity of urine (Le Gros Clark, 1958).

1.5 Ureteric pathology

The kidney, ureter and renal pelvis can be affected by many pathological conditions which range from congenital, neoplastic and inflammatory.

1.5.1 Congenital conditions

There are various congenital abnormalities of the upper tracts that may manifest in reflux or urinary stasis that may lead to stone formation and infection. The ureteric duct can be affected by growth failure that can cause renal agenesis; duplication; or positional abnormalities of the upper urinary tract. These result from abnormal development of the ureteric duct which originates from the mesonephric duct.

Agenesis

Renal agenesis is total failure of the ureteric duct to develop, when the ureteric bud does not fuse with the metanephros or when the ureteric duct forms a blind-ending diverticulum. Bilateral renal agenesis is a rare condition, however unilateral renal agenesis is more common (Magee, Lucey & Fried, 1979), about 1/1200 of screened children (Sheih *et al.*, 1990).

Duplication

A duplex collecting system develops when the ureteric duct elongates, the renal unit ascends and splitting of the ampullary bud. If splitting occurs in the early phase of nephrogenesis or along the mesonephric duct, two ampullary buds form and a second renal pelvis and ureters develop (Lenaghan, 1963). This condition is usually asymptomatic but may present with recurrent abdominal pain.

Ectopia

Two events occur to fuse the end of the ureter to the bladder: the ureteric duct migrates caudally along the mesonephric duct to attach to the bladder; and the vesicoureteric orifice migrates to its normal position at the trigone. However, if the ureteric duct develops close to

the bladder, then the vesicoureteric orifice may extend beyond the trigone laterally and caudally. The ectopic ureter is often inserted directly into the bladder wall with subsequent loss of the antireflux mechanism that results in vesicoureteric reflux (Pope *et al.*, 1999). In severe cases, the ureteric orifice extends beyond the bladder, and the ureter may can attach to the genital organs that develop from the mesonephric duct or attach to the urethra (Fernbach *et al.*, 1997).

Ureteric ectopia in males manifests with a single ureteric outflow, however, in females, there is a duplex system in about 80% of cases (Pope *et al.*, 1999). As the mesonephric duct provides the origin for the ureteric duct, abnormalities of the upper urinary tract can be associated with malformations of the genitourinary system such as prune belly syndrome, VATER syndrome and trisomy (Dewan et al. 1998).

Pelviureteric Junction Obstruction (PUJO)

This condition manifests in significant impairment of urinary flow from the renal pelvis to the ureter leading to hydronephrosis; it is mainly congenital and occurs in the paediatric population. However, this obstruction can also be due to other conditions such as inflammatory strictures, kidney stones, postoperative strictures and neoplastic growth (Karaca *et al.*, 1997; Lam, Breda & Schulam, 2007). There is manifestation of agenesis and cystic renal dysplasia in the contralateral kidney (Aslam, 2006). An increased risk of PUJ obstruction is also present in 15-20% of cases in congenital renal abnormalities (Dewan, Penington & Jeyaseelan, 1998). In paediatric patients, PUJ obstruction is frequently bilateral, whereas in adults it is usually unilateral. PUJ obstruction has also been reported in 16-20% of the population with extrinsic compression of this ureteric segment due to an aberrant lower pole vessel. Although the majority of PUJ obstruction cases could be due to a physical cause,

impairment of urine transport could also be functional as no obstructing lesion is always identified. (Ylinen et al. 2004).

Many studies have shown cellular and ultrastructural with increased interstitial collagen concentration. However, this finding may be secondary to the obstructive process (Gosling and Dixon 1978). In addition, reorientation of smooth muscle fibres and folds in the muscle or the mucosa layer have been proposed to occur with obstruction (Kaneto *et al.*, 1991; Starr *et al.*, 1992). Furthermore, innervation defects of the PUJ during obstruction has been found by immunohistochemistry (Harish *et al.*, 2003; Kuvel *et al.*, 2011), along with increased transforming growth factor beta 1 (TGF-β1) levels that indicate increased connective tissue deposition(Yang *et al.*, 2003).

Ureteric or Para-ureteric Diverticula and ureterocele

Ureteral diverticula are rare and can be congenital or acquired. They are sites of weakness in the ureteric wall and are located near the pelviureteric and vesicoureteric junctions (Rank et al. 1960). The acquired type can be secondary to obstruction or prolonged infection (Scarcello & Kumar, 1971; Summers, Keitzer & Hathaway, 1972). A ureterocele is a cystic dilatation of the distal segment of the ureter as it enters the bladder. This condition is more common in females and frequently associated with a duplex system (Rickwood *et al.*, 1992).

Megaureters

Large ureteric dilatation can be primary or secondary. Primary megaureters, when there is intrinsic smooth muscle defect, is characterised by a ureteric diameter of >7 mm and seen in the paediatric patients. It can be associated with ureteric obstruction or reflux, but many individuals have no reflux and non-obstructed ureters (King, 1980).

Ureteric Dysplasia

In ureteric dysplasia, the muscular layer has poorly structured myocytes with poor fibre organisation or absent muscle bundles. There is significant interstitial fibrosis with infiltrate of plasma cells and lymphocytes.

Ureteric Stricture

Ureteral strictures can be congenital or acquired secondary to trauma or inflammation. The aetiology of congenital strictures is unclear and could be associated with failure of ureteric recanalisation as the ureter is temporarily obliterated during foetal development (Ruano-Gil & Tejedo-Mateu, 1975; Cauchi & Chandran, 2005). Also, external compression of the ureter by foetal vessels can lead to poor development of the smooth muscles (Allen, 1970).

Acquired or secondary ureteric strictures can be due to trauma, ureteric inflammation due to infections, tuberculosis, radiation ureteric stones, vasculitis, ischemia and amyloidosis and endometriosis. Also, endoscopic procedures and pelvic surgery have an incidence rate for this complication ranging between 0.5-3% (Kubo *et al.*, 1996; Carl & Stark, 1997; Cholkeri-Singh, Narepalem & Miller, 2007).

Ureteric tumours

Primary tumours of the ureter such as transitional cell carcinoma, squamous cell carcinoma and adenocarcinoma, although rare, usually present with obstruction and stricture of the ureter. Also, extra-ureteric tumours may impinge on the ureter and obstruct by infiltration or metastatic spread (Chaudhary *et al.*, 2016).

Renal Pelvis and Ureteric Infection

One of the major causes of upper urinary tract infection is infection of the lower urinary tract, for example due to urinary tract obstruction and voiding dysfunction. Catheterisation, instrumentation and kidney stones also predispose to urinary tract infections. *Escherichia coli* is the most common pathogen of the urinary tract followed by species of *Proteus, Enterococcus, Klebsiella, Pseudomonas* and *Serratia*. Struvite stones are caused predominantly by *Proteus* infection (Stamm & Hooton, 1993). Mycobacterial infection of the ureter is usually secondary to an infected kidney which may result in the development of a ureteric stricture, obstruction and hydronephrosis. Candidal infection is the most commonly seen fungal pathogen and usually encountered in immune-compromised patients. Reports confirm that *Aspergillus* and *Coccidiosis*, may result in ureteral stricture formation (Wise, Talluri & Marella, 1999; Modi & Goel, 2007)

Kidney stones

Urolithiasis is a common disease that affects a large number of the population worldwide. It causes significant morbidity and in acute presentation, if not managed promptly, may lead to sepsis and risk of mortality. Kidney stones can affect all age groups with a prevalence rate of 4%-7% in women and 5%-12% in men. The rate of stone formation is associated with gender, age and geographical location (Romero, Akpinar & Assimos, 2010). It has frequently been reported that men have a higher ratio of forming stones in comparison with women. In children, kidney stones represent 1% of patients and have an almost 100% risk of urinary stone recurrence during their lifetime (Edvardsson *et al.*, 2013).

Dietary habits and environmental conditions play a significant role in urinary stone formation as well as medical conditions such as diabetes mellitus, gout and obesity (Chung, Chen & Lin,

2011; Marchini *et al.*, 2013). There are many consequences of kidney stones on the renal pelvis and the ureter, including obstruction, ureteric wall injury and stricture formation. Management of kidney and ureteric stones is influenced by stone size, location and stone composition and renal anatomy can influence the outcome of different treatment modalities (Mendez Probst, Denstedt & Razvi, 2009; Srisubat *et al.*, 2009; Straub, Gschwend & Zorn, 2010).

1.6 Ureteric physiology

Ureteric behaviour is dynamic and changes according to urine volume produced by the kidney and to the flow along its length (Constantinou, 1974). Pacemaker cells in the pelvicalyceal system depolarise spontaneously to generate action potentials that can conduct to adjacent smooth muscle to initiate contraction and the peristaltic wave to impel the urine bolus along the ureter to the bladder. The average number of ureteric contractions per minute in humans ranges between two to six contractions per minute (Ross et al. 1967). The urine production rate has a positive impact on the frequency and magnitude of contractions by the ureter. When diuresis is significant, the ureter may not have identifiable separate contractions and conducts urine in a continuous column of fluid (Boyarsky et al. 1981).

Ureteric smooth muscle has a broad length-tension relationship. Factors such as obstruction, diuresis and vesicoureteric reflux can affect ureteric smooth muscle fibre length and may influence the ability of the ureter to generate a propulsive force during peristalsis. The length-tension relationship is a primary property of skeletal, cardiac and smooth muscle. It expresses the relation between the force developed by the muscle when it is stimulated under isometric

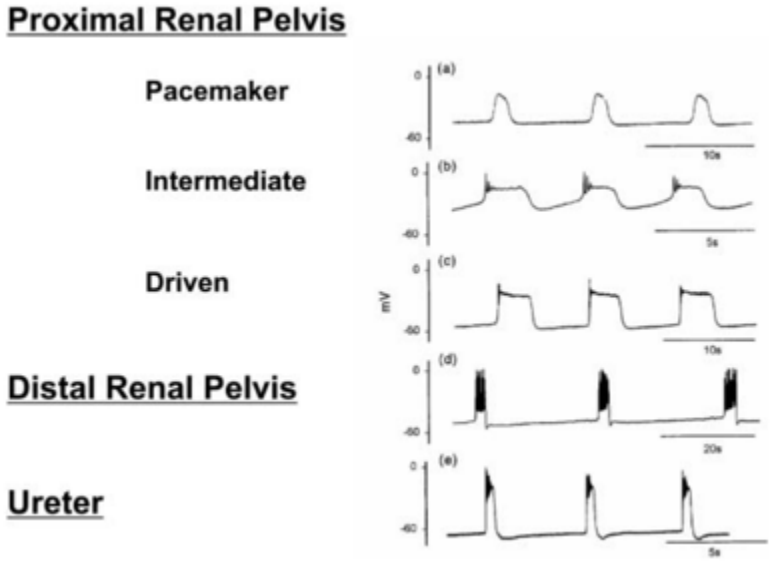
conditions and the resting length of the muscle at the time of stimulation. Mild degrees of dilatation is relatively well tolerated.

After severe and prolonged dilatation, ureteric function may remain permanently impaired even after correction of the causative factor. The ureter can compensate for muscle stretch over the peak of its length-tension; (Weiss et al. (1972) describe that stress relaxation in cat ureteric smooth muscle is exponential with the significant drop in tension occurring during the period immediately after stretch. Stress relaxation was associated with an increase in developed force (Weiss et al., 1972).

In the rabbit model Biancani et al. (1982) describe the effect of obstruction on ureteric circumferential force-length relation and show that after two weeks of obstruction; there is an increase in ureteric length by 24%, the outer diameter of the ureter by 100%, and the cross-section of the muscle region by 248%.

Also, the obstructed ureters show larger circumferential contractile forces than the control ureters. The peak active force of obstructed ureteric segments were approximately twice that of the control. The increase in the force generated was associated with a corresponding doubling of active circumferential stress (force/unit area) which implies that the force increment resulted from an increase in contractility and not a representation of muscle hypertrophy due to obstruction and reorientation of the muscle fibres. In this model, the ureter is not mechanically decompensated but instead undergoes alterations that increase contractility. Despite muscle hypertrophy and increased contractility, there is a decrease in the obstructed ureter's ability to generate the intraluminal pressures required for urine transport. The increase in ureteric diameter decreases the intraluminal pressure despite an increase in contractility following obstruction according to the Laplace relationship (Biancani et al., 1982).

In the upper urinary tract of many species, including humans and pigs, pacemaker cells – so-called atypical smooth muscle cells - located near the pelvicalyceal system can initiate ureteric peristalsis in the absence of an external stimulus (Lang *et al.*, 2006). These pacemaker spontaneously depolarise to threshold and initiate an action potential (Fig.4).



Chapter 1 - Fig 4. Action potentials recorded from proximal and distal renal pelvis of guinea pig ureter. (Adapted from Lang et al.1998).

In the ureteropelvic junction of the mouse, c-KIT–positive ICC-like cells have also been identified and showed high-frequency spontaneous transient inward currents that can act also as pacemaker potentials (Lang & Zoltkowski, *et al.*, 2007). It was hypothesised that in the absence of the above pacemaker drive these ICC-like cells could fulfil such a function and trigger contractions in adjacent smooth muscle cells in the ureteropelvic junction. Hence both atypical smooth muscle cells and ICC-like cells may play a pacemaker role in the initiation and propagation of pyeloureteric peristalsis (Richard J Lang *et al.*, 2006) (Lang & Hashitani, *et al.*,

2007). Whether such ICC-like cells are also present in larger animals and humans in not known.

The ureteric smooth muscle cell is the functional contractile unit of the ureter; they range between 250-400 micrometres (μm) in length and 5-7 μm in diameter. The generation of an action potential is the primary event in conduction of an electrical signal along the ureter, that in turn initiates a peristaltic wave of contraction. Conduction occurs at a velocity of 2-6 cm/sec (Ross 1972) and the electrical signal is transmitted from one smooth muscle cell to another via gap junctions.

1.7 Contractile activity

Contractile activity is determined by formation of cross-bridges between actin and myosin and is dependent on a transient increase of the intracellular Ca^{2+} concentration, $[Ca^{2+}]_i$, from less than 0.1 μM to 0.1-1.0 μM. Calmodulin (CaM) is a Ca^{2+}-binding protein which forms a $Ca^{2+}{}_4$-CaM complex to activate myosin light chain kinase (MLCK). MLCK catalyses the phosphorylation of the myosin light-chain (MLC) and permits contraction to occur (Yoshimura & Yamaguchi, 1997). Adenosine triphosphate (ATP) provides the energy for this process as it undergoes hydrolysis. Relaxation in phasic smooth muscles such as in the ureter is achieved by reduction of the $[Ca^{2+}]_i$, as described below. However, with smooth muscle, including ureteric smooth muscle dephosphorylation of MLC can also regulate the rate of relaxation through Rho-kinase-mediated and cyclic nucleotide-dependent intracellular pathways.

A rise of $[Ca^{2+}]_i$ for contraction comes from two sources: i) influx through L-type Ca^{2+} channels during the action potential (Brading et al., 1983; Hertle & Nawrath, 1989; Floyd *et al.*, 2008)

and release from intracellular Ca^{2+} stores, such as the sarcoplasmic reticulum (Burdyga *et al.*, 1998; Lang et al., 2002). An additional source of Ca^{2+} influx may also be via Na^{+}-Ca^{2+} exchange (Lamont, Burdyga & Wray, 1998).

Ca^{2+} is released from the sarcoplasmic reticulum (SR) of smooth muscle cells by an inositol 1,4,5-trisphosphate (IP_3)–induced release mechanism or by Ca^{2+}-induced Ca^{2+} release (CICR) (Somlyo & Somlyo, 1994). In addition to providing a source of Ca^{2+} for contraction, such an SR release can activate Ca^{2+}–sensitive K^{+} channels to modulate membrane excitability (Imaizumi et al., 1989; Carl et al., 1996). Relaxation results from a decrease of $[Ca^{2+}]_i$, in the region of the contractile proteins and can result from the uptake of Ca^{2+} into intracellular stores (Maggi, Giuliani & Santicioli, 1994) or extrusion of Ca^{2+} from the cell through Na^{+}-Ca^{2+} exchange (Burdyga & Magura, 1988).

1.8 The role of the nervous system

Contraction of ureteric smooth muscle depends on transmitter release from efferent nerves, augmented by subsequent impulse conduction between muscle cells. Ureteric peristalsis can also occur without neuronal stimulation via the pacemaker cells; this is seen in cases of renal transplantation when the ureter still exhibits peristaltic contractions (O'Conor, 1959). Also when denervation of the ureter occurs (Wharton, 1932), spontaneous activity in isolated *in vitro* segments has been observed (Finberg & Peart 1970) and regular antegrade peristalsis following reversal of a segment of the ureter *in situ* (Melick, et al. 1961). However, the literature indicates that the nervous system plays a modulating role in ureteric contractility.

The ureter is supplied by both noradrenergic-sympathetic and cholinergic-parasympathetic nerves (Duarte-Escalante 1969). Adrenergic and cholinergic nerve terminals have been

identified using electron microscopy within the adventitia, smooth muscle and submucosa of the human ureter (Schulman, 1975). With histochemical techniques, a more extensive cholinergic innervation was found at the pelvi-ureteric and vesicoureteric junctions in comparison with an adrenergic innervation (Duarte-Escalante 1969).

1.8.1 Sympathetic Nerves

Adrenergic receptors are present along the length of the ureter with a higher density in the intramural region (Weiss, Bassett and Hoffman, 1978; Morita *et al.*, 1994). The response to catecholamine release depends on the receptor that is activated; α-adrenergic receptors increase function, whilst β-adrenergic receptors reduce function.

In ureteric smooth muscle, α-adrenergic receptors predominate over β-adrenergic receptors, and for both receptor classes, subclasses $\alpha 1$, $\alpha 2$ as well as $\beta 1$, $\beta 2$ are found. Noradrenaline from sympathetic nerve terminals or adrenaline released from the adrenal medulla activates these receptors. Both alpha-adrenoceptor subtypes result in smooth muscle contraction, $\alpha 1$ by activation of phospholipase C and $\alpha 2$ by inhibition of adenylyl cyclase. In turn, $\alpha 1$-adrenoceptors predominate in the human ureter and are further subdivided pharmacologically into $\alpha 1A$, $\alpha 1B$ and alpha-1D-adrenoceptors (Sigala *et al.*, 2005). The density of $\alpha 1$-adrenoceptors is highest in the distal ureter as compared to the middle and proximal sections, and $\alpha 1A$, $\alpha 1D$ receptors predominate over $\alpha 1B$ at all levels.

The $\alpha 1$-adrenoceptors subtypes mediate their response via G-protein (G_q) coupled pathways, through phospholipase C (PLC). PLC activation results in the production of Inositol trisphosphate (IP_3) and diacylglycerol (DAG). These second messengers together release Ca^{2+} from intracellular stores and activate both voltage-dependent and voltage-independent Ca^{2+}

channels, as well as mediate activation of protein kinase C and phospholipase A2 and D, with arachidonic acid release and cAMP formation, that leads to muscle contraction. α-agonists will therefore increase the force of ureteric smooth muscle contraction; phentolamine, an α-adrenoceptor antagonist, reduces the force of contraction (Weiss, Bassett and Hoffman, 1978; Morita, Wada, Suzuki, *et al.*, 1987).

β1- adrenoceptors are predominant in the heart and adipose tissue and display an equal affinity for adrenaline and noradrenaline. β2-adrenoceptors are found in vascular, uterine and airway smooth muscle and exhibit a higher affinity for noradrenaline than adrenaline. β1, β2 & β3-adrenoceptors were found to be expressed in the normal ureter but lower expression in the dilated ureter (Shen *et al.*, 2017). The β-adrenoceptors are also coupled to G-proteins and subsequent intracellular second messenger systems. The β1-adrenoceptor is coupled to adenylyl cyclase via activation of stimulatory G-proteins (G_s). There is also evidence to suggest β-adrenoceptors form a link via a stimulatory G-protein to voltage-gated Ca^{2+} channels (Hieble *et al.*, 1995). Propranolol, a nonselective β-antagonist, increases the force of contraction by blocking β-adrenoceptors and promoting the stimulant effect of noradrenaline at α-receptors (Weiss, Bassett and Hoffman, 1978). Isoprenaline, a β-adrenoceptor agonist, decreases contractility in the ureter (Morita *et al.*, 1994; Danuser *et al.*, 2001). Salbutamol, a β2-adrenoceptor agonist, reduces the amplitude of ureteric contractions (Yamamoto 2000).

β-Adrenoreceptor agonists such as salbutamol is a selective β2-agonist used commonly as a treatment for acute exacerbations of asthma. β2-agonist induces smooth muscle relaxation and reduces bronchospasm. Salbutamol is also used for tocolysis in premature labour (Caughey & Parer, 2001) .

There is plausible evidence that the use of β-agonists in renal colic may have a relaxing effect on the ureter. Jung *et al.*, (2008) demonstrated the effect of isopreterenol on relaxing the ureter during ureteroscopy.

Cocaine inhibits the neuronal uptake of noradrenaline and therefore potentiates the effect of noradrenaline with a resultant increase in the force of contraction through α-adrenoceptors. β-agonists, such as adrenaline and isoprenaline, also facilitate Ca^{2+} influx through voltage-gated Ca^{2+}channels which may limit their relaxing effect on the smooth muscle.

α-blockers can relax the ureter in the presence of adrenaline, an endogenous ligand of β and α receptors; this finding led to an investigation of the role of β-adrenergic receptors in ureteric relaxation. Matsumoto et al. demonstrated the transcription of β1, β2 and β3-adrenoceptors mRNA acid in the human ureter and human ureteric relaxation is mediated by both β2 and β3 -adrenoceptor stimulation (Matsumoto *et al.*, 2013). β3-adrenoceptor agonists can potentially induce relaxation of the human ureter and they may have a role in the management of ureteric stones as medical expulsive therapy.

1.8.2 Parasympathetic Nerves

Ureteric smooth muscle cells do not exhibit discrete neuromuscular junctions and depend on the diffuse release of the neurotransmitter that may activate several cells.. Muscarinic receptors and acetylcholinesterase-containing neurons have been identified in the ureter (Prieto *et al.*, 1994) . Five muscarinic receptor subtypes (M1 –M5) have been demonstrated by immunohistochemistry in the human ureter, but polymerase chain reaction analysis has

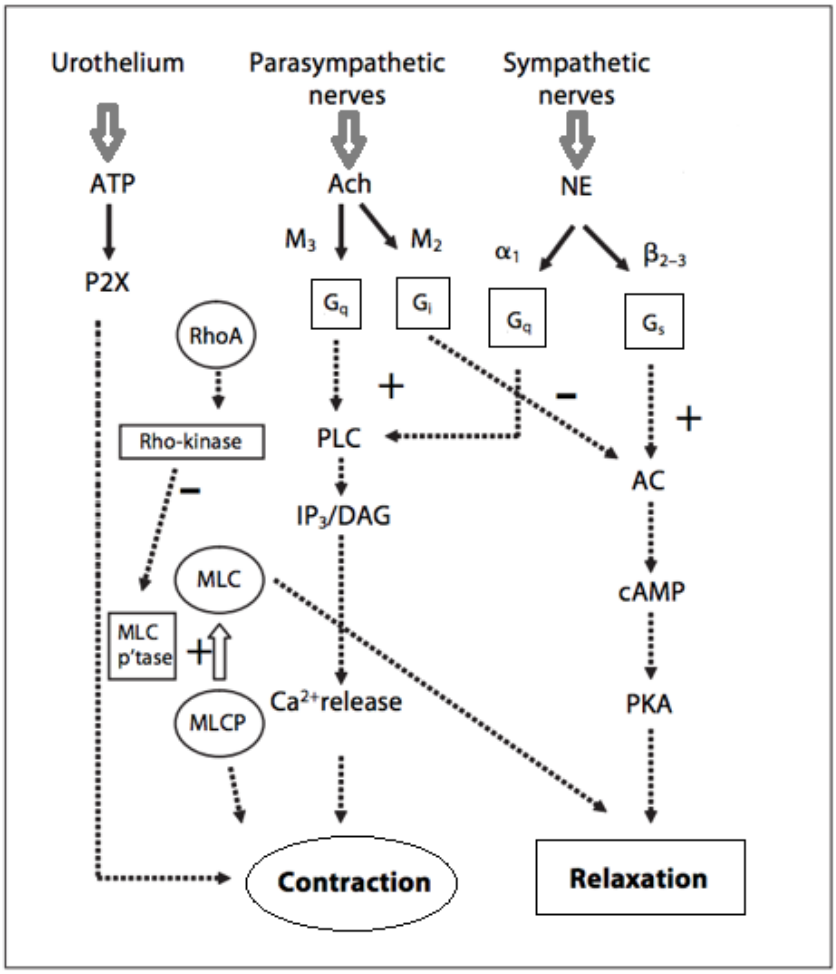
identified only M2, M3 and M5 receptor subtypes (Sakamoto, Suri and Rajasekaran, 2006c): the predominant subtype is M3.

Thus ureteric contractions occur by activation of M3 receptors via PLC activation and generation of IP_3 and DAG (Berridge, 1984). As with α1-adrenoceptors IP_3 will mobilise Ca^{2+} from the sarcoplasmic reticulum (Streb et al. 1983) and one action of DAG will be to increase Ca^{2+} influx across the cell membrane through a PKC-dependent pathway (Nishizuka et al. 1984) .An additional presence of muscarinic M1, M2 and M4 receptors in the porcine ureter has also been recorded (Londos, Cooper and Rodbell, 1981), but their role is less clear.

Overall muscarinic receptors do have a functional role. Carbachol, a non-selective muscarinic receptor agonist, has been used with isolated pig ureter (Hernández, García-Sacristán and Orensanz, 1995). Tomiyama et al. (2003) suggested that the contractions induced by carbachol in the isolated canine ureter were mediated by the M3 receptor. However, Roshani et al. (2003) demonstrated that mid and distal ureteric contractions were not modulated by muscarinic receptors in the porcine model. Furthermore, Tomiyama et al. (2004) demonstrated that carbachol reduced ureteric pressure and peristalsis in obstructed ureters of the anaesthetised dogs. The contractile response to carbachol thus varies between studies and one aim of this thesis it to clarify its action of pig ureter.

ATP is co-released from sympathetic and parasympathetic fibres and will act via a P2X1 receptor to cause Ca^{2+} influx and muscle contraction (Kennedy, 2015). ATP is present on the subepithelial sensory nerve fibres and stimulates purinoceptors (P2X and P2Y) in pigs and humans; it sends a stimulus to the pain centres located in the brain and initiates local reflexes (Burnstock, 2006).

Hence, ATP is considered to be a significant NANC component. ATP activates P2X1 receptors; ligand-gated cation channels that promote the influx of extracellular Ca^{2+} into muscle cells. The activation of P2Y2 or P2Y4 purinoceptors induces smooth muscle contractions via a PLC/IP 3 signalling pathway and causes release of intracellular Ca^{2+} (Burnstock, 2013). ATP is released due to mechanical stretch and also electrical field stimulation. The contribution of ATP release from neuronal sources is lower compared to non-neuronal origin. In the guinea pig ureter; ATP is released from the epithelium in a pressure-dependent manner which activates P2X 3 purinoceptors on the subepithelial sensory nerves causing pain and contraction (Knight *et al.*, 2002). A summary of autonomic responses, and the receptors mediating these responses are shown in fig.5.



Chapter 1 - Fig 5. Second messenger systems in the ureter.

Ach = Acetylcholine; ATP = adenosine triphosphate; NE = noradrenaline;

PLC = phospholipase C; IP 3 /DAG = inositol trisphosphate/diacylglycerol;

cAMP = cyclic adenosine monophosphate; PKA = protein kinase A;

AC = adenylate cyclase; MLC = myosin light chain; MLCP = myosin light chain phosphate; MLC = myosin light chain phosphatase, M2 -, M3 - and -receptors are coupled to G proteins, + = activation, – = inhibition. (Adapted from Canda et al. (2007).

1.9 The effect of the urothelium on ureteric contractility

Mastrangelo et al. (2003) demonstrated that the urothelium of the rat ureter produced nitric oxide (NO) which had an inhibitory effect on ureteric contractility. Furthermore, Mastrangelo & Iselin (2007) showed that the urothelium inhibited spontaneous contractions of isolated segments of the rat ureter and removal of the urothelium increased the stimulatory effect of carbachol, neurokinin A, vasopressin, bradykinin and angiotensin II. With ureteric segments where the urothelium is intact, cyclooxygenase inhibitors increased the stimulatory effects of neurokinin A, carbachol, vasopressin, bradykinin, and angiotensin II. Cyclooxygenase inhibitors did not exert any effect on the response to these agents in the ureteric segments with the urothelium removed. These findings may suggest that the inhibitory effect of the urothelium on ureteric contractility may be due to a urothelial cyclooxygenase agent such as prostacyclin. A similar reduction of bladder spontaneous contractions by the mucosa has also been recorded, but the mode of action in either tissue is not clear (Hawthorn *et al.*, 2000) .

1.10 Ureteric pharmacology

The investigation of ureteric physiology has always been closely related to pharmacology. Multiple agents have been used to investigate and manipulate the physiological response of the ureter. Ureteric colic had a significant focus due to the its clinical implications and painful

symptoms experienced by patients. The aim to develop a targeted therapy for ureteric spasms and contractions during colic has been sought since Laird et al. (1997) demonstrated that ureteric spasm causes pain.

The effect of nonsteroidal anti-inflammatory (NSAIDS) drugs has been extensively investigated due to their effect on prostaglandin production, which themselves are potent stimulators of ureteric smooth muscle contraction. NSAIDs have been successful in inhibiting ureteric contractility in many studies (Davidson and Lang, 2000). Both nonselective COX inhibitors and selective COX-2 inhibitors have an inhibitory effect on ureteric contractions (Chaignat *et al.*, 2008). In the clinical setting, patients who present with ureteric obstruction and inflammation, show up-regulation of COX-2 activity, and inhibition of COX-2 may relax the ureter in these conditions. Drugs such as indomethacin and Celecoxib can inhibit prostaglandin release in the ureter, even when COX-2 is upregulated (Davenport et al. 2010). These agents have a significant role in ureteric relaxation in obstructed and inflammatory conditions, but their clinical use can occasionally be limited due to their effect on renal function. When one kidney is obstructed, there is vasodilatation of the contralateral kidney to maintain glomerular filtration to some extent, and is driven by prostacyclin which is dependent on COX-2 activity. Blockade of this pathway by NSAIDs may therefore cause further deterioration in overall renal function (Hörl, 2010).

Ca^{2+} channel blockers and □-adrenergic blocking agents have been used in ureteric colic as a therapeutic modality, called medical expulsive therapy, to facilitate the passage of ureteric stones. These agents inhibit ureteric contraction and may reduce pain. Ca^{2+} channel blockers nifedipine and verapamil do not inhibit peristalsis but they can prevent ureteric spasm by inhibiting fast phasic contractions. Hertle & Nawrath (1984) showed that nifedipine increases

stone passage rate from 65% to 87%. Caravati et al. (1989) investigated the effect of nifedipine on pain control in patients with renal colic in a double-blind, placebo-controlled clinical trial. They evaluated the effect of nifedipine versus placebo in thirty patients but found no significant difference.

There is significant evidence that α-antagonists can relax the ureter and aid in the stone passage. α-adrenoceptor antagonists, selective or nonselective depending on their affinity for α1A and α1D receptors reduce ureteric spasm. Several randomised placebo-controlled trials have investigated the effect of Tamsulosin as a selective α-adrenoceptor blocker in comparison to nifedipine. The Tamsulosin group has shown a statistically significant ($p < 0.0001$) increase in stone passage rate in comparison with nifedipine (Dellabella et al. 2005).

Thus, α-blockers are currently favoured over Ca^{2+} channel blockers due to their efficacy and a low side effect profile. A meta-analysis by Hollingsworth et al. (2006) studied nine randomized controlled trials investigating the use of α-blockers and Ca^{2+} channel blockers. In comparison with the control group which are the patients who were not given these treatments; patients treated with nifedipine or Tamsulosin had an overall 65% increase in stone passage. Yilmaz et al. (2005) studied the difference in efficacy between Tamsulosin, Terazosin, and Doxazosin which are all α-blockers and found that they increased stone passage rate and all three agents were equally effective.

The SUSPEND trial which is a large UK clinical trial investigated the effectiveness of medical expulsive therapy in adults with ureteric colic in a multicentre, randomised, placebo-controlled trial.

Patients were randomised to tamsulosin 400 µg, nifedipine 30 mg, or placebo. The medication was taken daily for up to 4 weeks. The trial concluded that Tamsulosin 400 µg and nifedipine 30mg are not effective at decreasing the need for further treatment to achieve stone clearance in 4 weeks, for patients with expectantly managed ureteric colic (Pickard *et al.*, 2015). The SUSPEND trial has further supported the concept that medical expulsive therapy (MET) is not effective, but other clinical trials and metanalysis have shown favourable Hollingsworth et al. (2006) & Ye et al.(2018) and unfavourable results for MET , De Coninck et al. (2019). Further discussion about MET and the SUSPEND trial in chapter 4- section 4.1.9.

The effect of steroids on stone passage has also been investigated; as corticosteroids have an anti-inflammatory action and hence, may reduce the mucosal oedema in the ureter caused by an impacted stone and therefore may facilitate stone passage (Barnes, 2006). In a randomised clinical trial, 60 patients were randomised to α-blockers plus steroids, or steroids alone. However, the study did not show any difference in the overall stone passage rate. However, in the steroid group there was a more rapid rate of passage (72 vs 120 hr). Thus, Steroids may provide some benefit as expulsive therapy when combined with other medications, but not as a single agent (Dellabella, Milanese & Muzzonigro, 2005).

Many clinical trials investigating the benefit of αblockers have shown evidence they contribute to pain control in cases of obstruction and ureteric colic. Tamsulosin decreased discomfort and facilitated stone passage after treatment with shockwave lithotripsy, as well as reduced pain and colic in patients who developed persistent stone fragments (steinstrasse) after treatment (Gravina *et al.*, 2005; Resim, & Ciftci, 2005).

Another category of medication considered for a role in medical expulsive therapy is phosphodiesterase (PDE) inhibitors, as they have a significant effect on ureteric contractility (Stief *et al.*, 1995). Phosphodiesterase enzymes degrade cAMP and cGMP and blocking this process with PDE inhibitors leads to their accumulation, with subsequent smooth muscle relaxation. Relaxation occurs by activation of protein kinase A or protein kinase G and dephosphorylation of myosin light chain kinase. There are seven isoforms of PDE inhibitors and PDE 4 inhibitor - rolipram has the most significant effect on ureteric relaxation (Becker *et al.*, 1998). Nitric oxide (NO) is an inhibitory neurotransmitter in the ureter and may have a role in ureteric relaxation in humans (Stief *et al.*, 1996). *In vitro*, NO inhibited ureteric contractions in rats (Iselin *et al.*, 1997). NO may play a specific role at the ureterovesical junction, where it has been proposed that NO has a regulatory role on the anti-reflux mechanism of this ureteric segment (Yucel and Baskin, 2003). Multiple agents are being investigated to aid in ureteric relaxation; histamine antagonists, 5-HT receptor antagonists, Vasoactive intestinal polypeptide, neuropeptides, Calcitonin gene-related peptide, Neuropeptide Y, and agents which affect the Rho Kinase pathway (Venkatesh *et al.*, 2005).

Carbachol has been used in several investigations involving the ureter and it is a synthetic choline ester, a positively charged quaternary ammonium compound and a Para-sympathomimetic; It mimics the effect of acetylcholine on muscarinic and nicotinic receptors. Carbachol has also been used to stimulate micturition by contracting the detrusor muscle; this drug may cause hypotension, bradycardia, nausea, vomiting, bronchospasm, and abdominal cramps. Carbachol induces ureteric contraction during investigation on human, pig and sheep ureters in vitro (Hernández, García-Sacristán & Orensanz, 1995; Nyirády, Cuckow and Fry, 2008).

Atropine is a naturally-occurring alkaloid isolated from the plant Atropa belladonna. Atropine functions as a sympathetic competitive antagonist of muscarinic cholinergic receptors. It thereby abolishes the effects of parasympathetic stimulation. This agent may induce tachycardia, inhibit secretions, and relax smooth muscles. The effect of atropine on ureteric contractions has been described as inhibitory in pigs and dogs (Tomiyama et al. 2004; Tomiyama et al. 2003).

1.11 The pig urinary tract

The pig urinary tract provides a good experimental model for the human tract with much resemblance in terms of morphology and physiological function (Sampaio, Pereira-Sampaio & Favorito, 1998).

The adult domestic pig kidney weighs between 98-280 grams with a length of 12-13 cm, including the cranial and caudal poles (Sisson, 1975; Sampaio and Mandarim-de-Lacerda, 1989). One study (Sampaio, Pereira-Sampaio & Favorito, 1998) reported two different structure of the pig renal system; the first type is characterised by major cranial and caudal pole calyces (40%) and the second is mainly comprised of calyces in the midpart of the kidney that drain directly into the renal pelvis (60%). The human kidney mainly (62%) is formed of major superior and inferior calyces (Sampaio and Mandarim-de-Lacerda, 1989). Wolf et al. (1996) studied the comparative micro-anatomy of the domestic pig, mini-pig, human ureter and other animals including rat, rabbit, dog and sheep. Of interest, the sheep ureter was most similar to the human ureter but was 45% smaller. The domestic pig and the mini-pig had a similar ureteric cross-sectional thickness of the human ureter but only the domestic pig had the same cross sectional area. Overall, to study methods that assist in the expulsion of

ureteric stones the domestic pig ureter was considered most suitable.

1.12 Methods of investigating the ureter

1.12.1 In Vitro Methods

Investigation of different animal and human ureteric segments has been useful to develop a conceptual and functional understanding of ureteric physiology in general and the effect of multiple medications that may manage ureteric conditions. One of the most painful and common conditions is ureteric stones, and a significant number of publications have investigated the effect of drugs on stone passage. These have been carried out on pig, rabbit, sheep, guinea pig, rat and dog tissues; with pig and sheep ureters histologically more similar to human ureter (Wolf *et al.*, 1996). Ureteric segments are placed in a buffered solution maintained at 4°C until the time of experimentation, to maintain the tissue function. During experiments segments are suspended in a jacketed organ bath maintained at 37°C and tied to an isometric force transducer, whilst irrigated with a buffered physiological solution, usually gassed with a 95% O_2/5% CO_2 mixture. At the outset of the experiment, a resting tension is applied to the preparation and allowed for 30-60 minutes. Spontaneous phasic contractions represent a healthy, functional preparation. These phasic contractions allow two main variables to be measured, amplitude and frequency of contraction (Lang *et al.*, 2006). In addition, drugs added to the superfusate may also generate tonic contractions or relaxation. Overall, concentration-response curves to drugs may be compiled by measurement of their effects on the above contractile responses. Useful drug interventions include the muscarinic agonist carbachol and the high-K solution to depolarise the muscle (Van Mastrigt, 1985).

1.12.2 In Vivo Methods

Many studies have investigated ureteric physiology in vivo using animal and humans models describing the peristaltic process and the response to physiological and pharmacological changes. Intraluminal methods include intra-ureteric pressure measurement and endoluminal ultrasound, but extraluminal ultrasound has been used during laparoscopy (Bagley et al. 1994). The contraction amplitude generated by the ureter during in vivo investigations is more difficult to record accurately, whereas contraction frequency is easier. However, intraluminal methods have numerous limitations: such as antegrade or retrograde ureteric may affect the normal physiology of ureteric contractions. (Kondo 1970). The choice between animal species and humans comes at the price of convenience versus accuracy. Human tissue is more difficult to acquire, but animal tissue is not always representative of human ureteric function.

Many extra-luminal investigations have been done using dogs and pigs, but these methods are quite invasive and require a surgical procedure, either through an open or laparoscopic approach. Moreover, extraluminal methods are expensive, more complicated and may not always be possible in humans. Ureteric contractions in vivo have been recorded for a short period but functional changes over a significant period were not found (Tillig *et al.*, 2004). Moreover, other factors such as anaesthetic medications and analgesia, and the state of hydration can affect ureteric contractile activity (Osman *et al.*, 2009). Overall the in vitro approach is useful to establish fundamental aspects of ureteric physiology and will be adopted in this thesis.

1.13 Hypothesis, aims and experimental objectives

The dynamic contractile properties of the ureter allow it to maintain a continuous conduction of urine from the kidney to the bladder. The changes exhibited by the ureter to obstruction and various perturbations present a complex and poorly-characterised adaptation.

Several studies have investigated the compensatory and adaptive behaviour of the kidney during ureteric obstruction. Moreover, the ureter has been characterised in terms of its response to several drugs. However, the response of the ureter to external stimuli, such as the presence of a stone in the lumen, are not well-characterised.

The hypothesis to be tested in this thesis is that:

The ureter adapts its functional behaviour to obstruction of its lumen.

The aims of this thesis are;

1. To compare the behaviour of the basal and pharmacologically manipulated ureteric contractions of different segments of the ureter.
2. To describe histological variations of the different segments of the ureter.
3. To determine the ureteric response to obstruction in an *in-vitro* model.

The experimental objectives were carried out with an in vitro approach with pig ureter:

1 Describe the basal contractile activity of different segments of the pig ureter

2 Characterise the effects of carbachol and high-K solutions of contractile activity

3 Describe the histological structure of different segments of the pig ureter

4 Measure the functional activity of different segments of the obstructed pig ureter

Chapter 2

Investigation of the basal spontaneous activity of the pelvicalyceal system, renal pelvis, upper, middle and lower ureteric segments in a porcine model.

2.1 Introduction

The contraction of the ureter along its length and the propagation of peristalsis presents an interesting phenomenon. The initiation and propagation of the peristaltic wave along the different ureteric segments has not been sequentially and completely described. The continuity of peristaltic propagation poses an essential question; is there a specific role for each of the ureteric segments during peristalsis? The ureter has been previously described as an organ which contracts as a syncytium (Aragona *et al.*, 1988). If that is true, then each segment would behave in a similar fashion to all the other segments regardless of its position in the upper urinary tract.

As mentioned earlier in chapter one of this thesis, the ureter is not merely a tube or conduit that simply vermiculates, it rather demonstrates complex and changing behavior. In order to understand the degree of ureteric physiological complexity, various inferences can be deducted from the relationship between its morphological structure and contractile behaviour in *ex-vivo*, *in-vivo* or *in-vitro* studies.

In order to further understand the dynamics of peristalsis; the aim of this study was to evaluate the following objective which is:

To measure and compare the non-stimulated basal contractile behaviour of the different segments of the ureter.

2.1.1 Ureteric peristalsis

As urine flows from the upper urinary tract to the lower segments of the ureter, the peristaltic wave goes through different stages that is influenced by ureteric frequency and amplitude of contraction. Morita et al. show that the frequency of contraction at the pelvicalyceal system (PCS) and the renal pelvis (RP) is greater than that in the upper ureter and there is a subtle electric block at the pelviureteric junction (PUJ) (Morita, Ishizuka & Tsuchida, 1981). Urine accumulates in the renal pelvis and as the pressure increases in the collecting system, the urine is expelled into the upper ureter which is believed to be in a collapsed state (Griffiths & Notschaele, 1983). The pressure generated by the ureter propagates the urine to flow forwards; the kidney is protected by the PUJ by preventing the back flow of urine. As the rate of flow increases, the PUJ dilates and there is a constant flow ratio with 1: 1 between the PCS, renal pelvis and the upper ureter.(Constantinou and Hrynczuk, 1976) (Yamaguchi & Constantinou, 1989)

In pelviureteric junction obstruction, the areas of narrowing due to mucosal folds (Takeyama & Sakai, 2007) or a valve like structure (Maizels & Stephens, 1980) may hinder this propagation and obstruction develops. The PUJ may not be obstructed anatomically, but an abnormal peristaltic propagation may occur, leading to functional obstruction as the urine flow from the kidney may be greater than the ability of the PUJ to allow the effective passage of urine. In this situation, a ureteric catheter can be introduced through the PUJ or during ureteroscopic examination of the ureter in patients with signs and symptoms of PUJ obstruction, yet they are found to have a patent PUJ segment during the examination.

2.1.2 PUJ obstruction

There are multiple possible aetiologies for PUJ obstruction but intrinsic obstruction due to fibrotic and adynamic stenotic segment of the ureter is the most common aetiology in about 75% of cases (Woodward & Frank, 2002), that results in failure of peristalsis producing an incomplete, functional obstruction. Other causes include crossing vessels (20%), peripelvic fibrosis, abnormal ureteric insertion, fibroepithelial polyps and anatomical variants, such as retrocaval ureter, horseshoe and duplex kidneys (Hashim and Woodhouse, 2012).

Alternatively, crossing lower-pole vessel/s cause entrapment of the ureter and hence obstruction to urine flow. The abnormal high insertion of the ureter into the renal pelvis may alter the configuration of the ureter and impair the drainage of urine. Renal ectopy or malrotation of the kidney can be potential causes for intermittent obstruction of urine flow (Hindryckx & De Catte, 2011).

A secondary cause of PUJ obstruction can be due to failed prior surgical intervention for PUJ obstruction or an impacted ureteric stone that has caused intrinsic ureteric and or periureteric fibrosis and scarring. These abnormalities impair urine drainage and result in elevated intrarenal pressure, dilatation of the collecting system, and hydronephrosis (Rogers & Hasan, 2013).

There is evidence confirming smooth muscle apoptosis and defective neural development in the pathogenesis of congenital ureteropelvic junction obstruction which may affect peristalsis through the PUJ (Kajbafzadeh *et al.*, 2006). Murnaghan (1958) attributes the functional obstruction or abnormality to an altered configuration in the layout of the muscle bundles. Also, Foote et al. (1970) describes a decrease in the muscle bundles at the PUJ.

In an electron microscopy study, Hanna (1978) found a decrease in the muscle fibres in severe PUJ obstruction, and also abnormalities in the muscle layout of the renal pelvis and disruptions between the intercellular relations at the PUJ.

The effect of mechanical obstruction caused by a crossing vessel at the PUJ may lead to subsequent histological changes and ischemic related fibrosis of smooth muscle as shown in the specimens obtained during Pyeloplasty. The stenotic segments demonstrated severely impaired contractility in comparison with pre and post stenotic segments. (Portincasa et al. 2006)

The Pelviureteric junction provides a reflective example of how a ureteric segment may contribute to the overall success of the peristaltic process. If that is the case with one segment, then the next question would be; how do other segments contribute to peristalsis and the propagation of urine?

2.1.3 Peristaltic propulsion

The propagation of the peristaltic wave has been described by many authors and a depiction of how the process may occur is described by Griffiths & Notschaele (1983); During normal physiological flow, urine accumulates in the renal pelvis; with the rise in the renal pelvic pressure, urine is then passed onto the upper ureter which is believed to be in a collapsed state and that the urine bolus is propelled through the coaptation of the ureteric wall. A ureteric contraction is described to be originating in the most proximal portion of the ureter and propels urine forward towards the distal ureter. (Woodburne & Lapides, 1972).

Yin & Fung (1971) & Weinberg (1974) described that the bolus of urine is propelled forward by ureteric contraction that creates intraluminal pressure that aids in the propelling process.

The resting pressure in the ureter is approximately 0 to 5 cm H_2O and the ureteric contractions raise the pressure to a level between 20 to 80 cm H_2O. The number of ureteric contractions per minute ranges from two to six times Kiil F. (1973) Ross et al. (1972). At the level of the Vesicoureteric junction (VUJ), urine enters the bladder and is prevented from retrograde reflux back into the ureter. The urine bolus creates enough pressure to traverse the VUJ and the force of that contraction dissipates where the ureter joins the bladder.

During regular flow conditions, the maximum transport capacity of the ureter is more than the bolus of urine being conducted from the upper to the lower segments. As the ureter is a tubular structure, it can transport a set volume of fluid per unit time.

Whitaker (1973) in his standard perfusion studies described the flow characteristics in the ureter. At extremely high flow rates, the ureteric walls do not coapt and a fluid column is formed to transport the urine rather than regular boluses of urine being propelled. Urinary stasis occurs when there is inadequate transport which could be due to high inflow per unit time or low outflow per unit time. Ureteric dilatation can be the result of this phenomenon.

The ureter has the ability to adapt to the different rates of inflow by changing its dimensions to accommodate the varying volumes of urine.

Pathologic conditions can affect this adaptability of the ureter and may result in delayed urine transport. Intraluminal obstruction leads to increased intraluminal pressure, the ureter compensates by increasing its diameter to partly reduce the intraluminal pressure and perhaps to protect the kidney, if this condition persists, it may result in inefficient urine transport with potentially deleterious effects on the kidney. (Griffiths & Notschaele, 1983)

The ureter can also be affected by extraluminal compression or conditions that may increase the wall tension as in retroperitoneal fibrosis. The Laplace equation (Boyarsky, S., Tanagho, E. A., Zimskind 1971) demonstrates the relationship between the factors that may affect the

intraluminal pressure which is related to the wall tension and tubular diameter by the relationship:

$$\text{Pressure} = \frac{\text{tension x wall thickness}}{\text{Radius}}$$

Laplace's law shows that as the radius of a vessel increases, the intraluminal pressure declines for a given wall tension. In ureteric obstruction, either due to intraluminal or extraluminal causes; there is a change in the flow dynamics of urine. In the pre-obstructed segment of the ureter; there may be a dilatory adaptation of the ureteric wall causing hydroureteronephrosis. The change in the ureteric wall caused by the dilatation and obstruction will affect the flow dynamics of the urine and ureteric contractility. As the change in the ureteric wall tension may change from one ureteric segment to the next, i.e. from the segment above the obstruction to the obstructed segment, there is an expected change in flow characteristics. There may be a transient compensatory ureteric contraction (spasm) to overcome the obstruction, but then gradual muscle fatigue will ensue. The resultant change in the flow of urine and especially in segments with partial obstruction over time may gradually affect renal function (Basile, Anderson & Sutton, 2012). Understanding the contractile characteristics of the different ureteric segments may provide an insight into ureteric adaptability and compensatory mechanisms.

Lee et al. (1998) demonstrate that the histological composition dilated ureters that occur in obstruction and megaureters have different amounts of type I and type III collagen. As the peristaltic wave propagates to the VUJ, the bolus of urine under normal flow conditions exerts the pressure necessary to overcome the intravesical pressure and the bolus of urine traverses

the VUJ into the bladder. (Griffiths & Notschaele, 1983). The mechanism of urine transfer across the VUJ involves the creation of pressure and thrust of the urinary bolus to traverse the VU junction which does not relax to allow the passage of urine (Weiss & Biancani, 1983). During the ejection of the urine bolus into the bladder, the distal ureteric segment moves within its sheath in a projectile and retractile fashion, which decreases the resistance to flow at the VUJ and facilitates the process of emptying the urinary bolus into the bladder. (Blok *et al.*, 1985). During obstruction at the VUJ and raised intravesical pressure or excessively high flow rates that exceed the ability of the VUJ to allow efficient fluid transport, there is an impediment of the transfer of the urinary bolus into the bladder. In this instant the urinary bolus cannot traverse freely into the bladder and there is rise in the pressure exerted by the bolus which may exceed the pressure in the contractile wave. The contractile wave usually encircles the ureter but during these changes in pressure of the urine bolus, the contractile wave loses the ability to occlude the ureter and there is retrograde flow of urine so that only a fraction of the bolus of urine passes into the bladder.

In the event of ureteric lack of contraction or weak contractions, Griffiths & Notschaele (1983) demonstrate theoretical evidence that this phenomenon would lead to impaired transport of the bolus of urine across the VUJ. Inherent defect in the ureteric contraction may lead to a wider, weakly contracting ureter that may become less effective to deliver the urine bolus to go across the VUJ which should have low resistance in order to allow urine transport. Tanagho (1968) describe that at the VUJ, the resistance to flow may be affected by the forces of muscle contractions at the trigone. Other authors attribute this flow resistance to the detrusor pressure (van Duyl & Coolsaet, 1982).

Schick and Tanagho (1973) demonstrate that gravity may aid in the transport of urine through the VUJ when the ureter is in the erect position, especially in patients with dilated upper

tracts. Also, an interesting notion has been described in patients with wide upper tracts and urinary retention, bed rest may be deleterious to renal function (Holden *et al.*, 1984).

The anatomy and function of the VUJ have been debated for a long time. Three schools of thought propose their ideas about the antireflux mechanism at the VUJ. The first theory suggests that the VUJ is thought to be governed by an inactive valve mechanism dependent on the oblique direction of the terminal ureter as it enters the bladder and the length of the intravesical ureter. length (Paquin, 1959; Juskiewenski *et al.*, 1984). The second theory proposes that the VUJ possess mixed active and passive valvular action. Also, in addition to the anatomical factors, the distal ureter displays antireflux ureteric peristaltic activity so that contraction of the ureter can prevent the retrograde passage of urine (Hutch *et* al. 1961; Roshani, *et al.*, 2000; Roshani, Dabhoiwala, *et al.*, 2000). The third theory affirms that the VUJ is thought to be acting as a sphincter. Noordzij and Dabhoiwala, (1993) proposed a sphincteric function for the VUJ, which could be enhanced by the intrinsic musculature of the trigonal region of the bladder, which complements a merely passive antireflux mechanism.

Multiple studies investigated the flow characteristics through the VUJ using doppler ultrasound and delineating the flow wave and characteristics. Considering all the features observed in multiple Doppler US studies of the ureteric jet, it is proposed that the human VUJ tends to can act as a functional sphincter (Leung & Metreweli *et* al.,2002). There is a monophasic ureteric peristaltic wave demonstrated by M-mode study of the ureter while the waveform of the ureteric jet originating from the VUJ demonstrates a more complex pattern. As there is an alteration of the flow pattern as the waveform of the ureteric jet is modified, it is proposed that an active sphincter mechanism is probably present at the VUJ (Leung & Metreweli et al., 2002). As the VUJ shows sphincteric activity, different patterns of the

ureteric jet can be identified under various physiological and pathological conditions with infrequent modifications (Leung *et al.*, 2007).

2.1.4 The investigation of peristaltic propulsion

Ureteric peristalsis has been described in different contexts but the process of peristaltic initiation and propagation has not been clearly described in a sequential manner along the different parts of the ureter and the upper urinary tract.

Constantinou (1974) described the initiation of the peristaltic wave from the renal pelvis, Hannappel et al. (1982) & Tsuchida et al. (1981) describe the role of the pelvicalyceal system in the initiation and propagation of peristalsis.

As the urine passes from the kidney into the pelvicalyceal system (PCS) and the renal pelvis (RP), it has been described as mentioned earlier that the urine accumulates in the upper segments and creates pressure and through that pressure it is expelled into the upper ureter. This may demonstrate an explanation of the process but it is a passive process of urine transport. There is a discrepancy in understanding how the process occurs as the upper segments i.e. the PCS and RP are contracting segments and may have an active role in the propelling urine through the PUJ. The same concept applies to the rest of the ureter and whether other segments along the length of the ureter contributes to the peristaltic wave. In the literature, there is no description of how each segment contributes to the peristaltic process and how each segment may play a role in the delivery of urine to the next segment.

The description of the PUJ obstruction or in instances when the PUJ may not be physically obstructed but may be functionally obstructed as during surgery or intervention, the PUJ may be open but still the urine transport is inadequate. This segmental failure may not be the only part of the ureter that is not contracting and leading to urinary stasis but rather a cumulative

effect of other parts or the rest of the ureter. The flow of urine through the two junctions at each end of the ureter, the PUJ and the VUJ, are interesting phenomena, in trying to understand the relationship between flow and pressure. Also, the contractile force created by the ureter to deliver the urine bolus through both junctions may reflect an intricate interaction between different physiological phenomena. The ureter is not a uniform structure as demonstrated earlier in chapter one, it has the ability to coordinate variable degrees of pressure and flow along its different segments and in response to different conditions. In this chapter, a detailed investigation of the ureter will demonstrate the different functional characteristics of each segment and allude to the initiation of the peristaltic wave.

2.1.5 Hypothesis

The peristaltic process starts at the pelvicalyceal system and the renal pelvis and continues along the length of the ureter to propel the urine bolus into the bladder. We hypothesize that there are physiological differences in the contractile properties of each segment that allows it to contribute to the peristaltic process.

2.1.6 Aims

The aim of this study was to investigate the basal spontaneous activity of the different segments of the upper urinary tract and the potential role of each segment in the peristaltic process.

2.1.7 Objectives

To examine the basal spontaneous activity of the upper tract and ureteric segments; the Pelvicalyceal system (PCS), Renal pelvis (RP), Upper ureter (UU), Middle ureter (MU) and to the Lower ureter (LU) and evaluate the differences and similarities in the contractile behavior of each segment in terms of the amplitude and frequency of contraction.

2.2 Methods

The methods described here refer to the experiments described in Chapters 2-4. Experiments in Chapters 2 and 3 measure spontaneous contractions and the influence of the cholinergic receptor agonist carbachol. Chapter 4 describes experiments in which the influence of an artificial obstruction placed in the ureteric lumen affects spontaneous contractile function.

2.2.1 Tissue retrieval

The pelvicalyceal system, renal pelvis, upper, middle and lower ureteric segments were retrieved from the urinary tract of freshly slaughtered female pigs aged between 6 and 8 months. The urinary tract was collected intact in its entirety. This was to ensure the integrity of the tissue and for dissecting the specimens in the lab.

The abdomen of the animal was dissected open and the intestines were retracted to one side and the posterior peritoneum was approached. The kidney and ureter were identified and protected. The kidney was dissected free and the renal artery and vein were dissected. The ureter was separated from the retroperitoneal attachment and followed down to the bladder. The bladder was cut at the urethra and then dissected from its attachments making sure the ureter was protected. The specimens, once free were kept in cold, buffered Krebs bicarbonate solution for transport in sealed containers on ice.

The Krebs buffer included 118.4 mM NaCl, 11.7 mM glucose, 24.9 mM $NaHCO_3$, 4.7 mM KCl, 1.9 mM $CaCl_2$, 1.15 mM $MgSO_4$ and 1.15 mM KH_2PO_4.

2.2.2 Tissue preparation

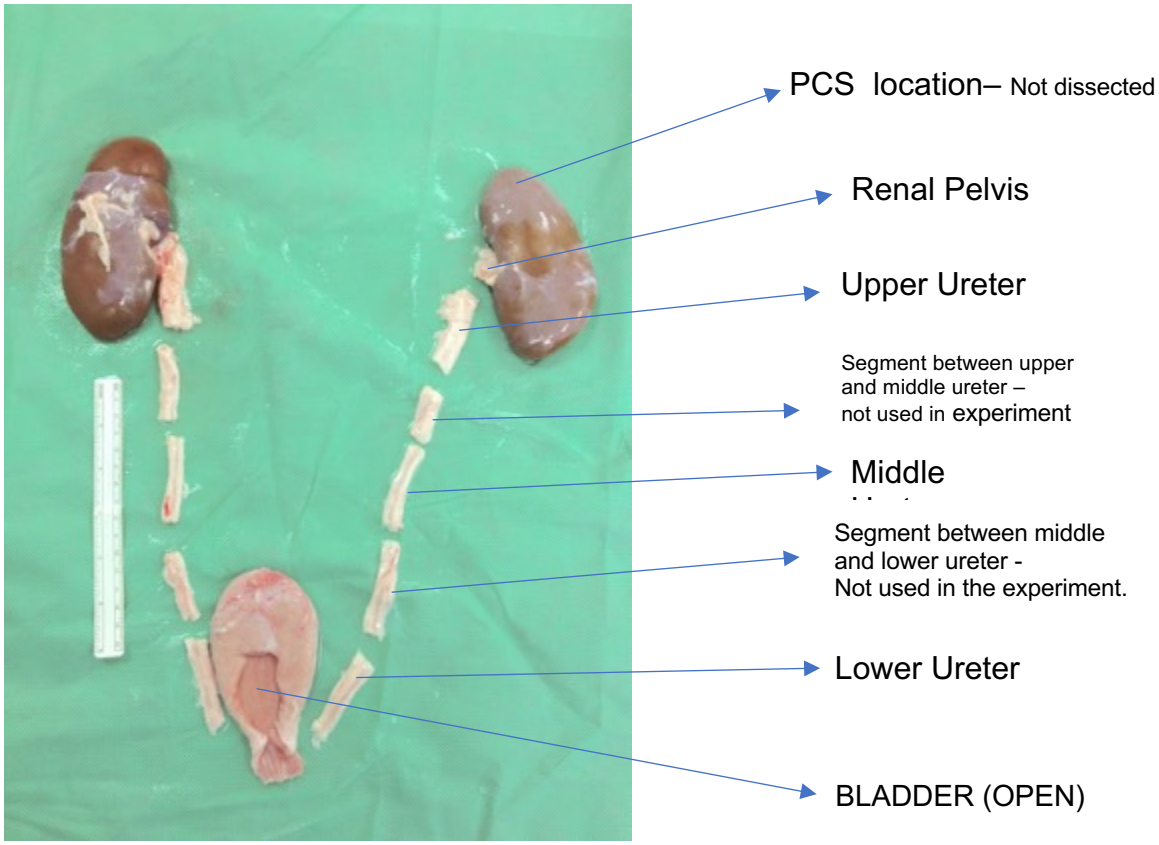
In the lab, the specimens were gently dissected as per the following protocol.

The upper urinary tract was divided into five segments; the pelvicalyceal system (PCS), renal pelvis (RP), upper ureter (UU), middle ureter (MU) and lower ureter (LU). based on its morphological aspects, anatomical location and length. The pelvicalyceal system and the renal pelvis were obtained through the dissection of the kidney from the lateral aspect to evenly expose the pelvicalyceal system PCS and the RP.

Segments of the PCS were dissected from the region of the renal calyces, the tissue at this level is thin and quite adherent to the underlying renal tissue and it converges towards the renal pelvis. At this point, the tissue is separated to make sure the renal pelvis is not part of this specimen. The renal pelvis segment is taken from the part of the intrarenal pelvis with a cutoff point at the exit from the kidney to avoid taking part of the upper ureter. At this level the tissue is thicker, with less visible blood vessels and has a white creamy color and there are fine converging lines towards the ureter with a clear demarcation between the PCS and the renal pelvis. The upper ureteric segment is taken below the renal pelvis after it converges into the UU, to ensure that tissue from the renal pelvis is not incorporated into the UU segments, the tissue is transected about a centimeter below the renal pelvis. The caliber of the ureter changes along its length with wider renal pelvis, narrower upper ureter and further smaller caliber of the middle ureter. The middle ureteric (MU) segments were taken about 10cm below the upper ureter to make sure that the UU has ended and the MU segment began and the caliber delineation of the MU was taken into consideration as it became narrower

and with less muscle mass in comparison with the upper ureter. The lower ureteric segments were taken one centimeter above the intravesical segment of the ureter above the vesicoureteric junction, this is to make sure there is no bladder muscle fibers involved in the specimen.

The dissection method described above for tissue retrieval was applied to ensure consistency and practicality of obtaining the specimens, not only considering the length of the ureter but also the morphological differences between the segments. All segments were dissected gently to preserve the morphological and the physiological integrity of the tissue. The segments were then cut into longitudinal strips of tissue ranging between 2.5 to 3 cm to have a uniform tissue setup for all segments except for the PCS segments as they were smaller in size due to its anatomical and morphological nature and the segment length ranged between 1 to 1.5 cm – Fig 1.



Chapter 2 - Fig 1 - Pig urinary tract.
The ureteric segments are dissected and the anterior bladder wall is partly exposed.
Scale - 15cm length.

2.2.3 In vitro experiments – measurement of spontaneous activity

The ureteric segments were mounted in of water-jacketed glass organ baths by attaching the lower end of the segment to an anchor at the bottom of the organ bath. The upper end of the preparation was attached via a silk suture that was introduced through the tissue and tied to an isometric force transducer. The segments were perfused with Krebs bicarbonate solution (section 2.2.1), maintained at 36±1°C, and gassed with 95% O_2 / 5% CO_2 to maintain adequate oxygenation and maintain pH at 7.40±0.05. Segments were equilibrated under a resting tension of 1.5–2.0 g for 60 min. Tension was monitored via isometric force transducers (Pioden Controls Ltd, UK) and a Powerlab data acquisition system using LabChart software (ADInstruments, UK). The Krebs solution was changed every 15 to 20 minutes during an

equilibration period and separately warmed before addition to avoid fluctuations of temperature.

When contractile activity was consistent for basal spontaneous activity was recorded without any addition to the superfusate. The frequency and amplitude of contractions were recorded for subsequent analysis without any additions to the superfusate. Spontaneous activity of all the segments, i.e. the pelvicalyceal system (PCS), renal pelvis (RP), upper ureter (UU), middle ureter (MU) and lower ureter (LU) were recorded for 60 to 90 minutes.

Subsequently, Interventions with carbachol when required were by addition to the superfusate from an aqueous 10mM stock solution to achieve the required final concentration (10 μM). The concentration was chosen from the literature as that which achieved a maximum effect but allowed recovery of contractile changes on its removal (Nyirády, Cuckow and Fry, 2008).

2.2.4 In vitro experiments – measurements with artificial obstruction

An artificial obstruction, to mimic a ureteric stone, was introduced into ureteric segments as a rounded ball of aluminium foil. The obstruction was uniformly, 6 mm diameter that would prevent irritation of the mucosa. The size was chosen to fit comfortably into the lumen and obstruct it without causing excessive dilation. The size was also guided by a literature review of stone sizes that had a 25% to 50% possibility of spontaneous passage in humans (Hollingsworth *et al.*, 2006). The similarity of human and pig ureteric dimensions was another factor that made this choice of animal model particularly suitable. The preparation was mounted in the organ bath a little differently to allow potentially passage of the obstruction.

In his case the lower end of the ureteric segment was attached at one side to the anchoring needle to allow the lumen to remain patent.

With these experiments, the ureter was divided from the kidney below the level of the renal pelvis based on the morphology and its anatomical location where the funnel-shaped renal pelvis converged to form the upper ureter. This dissection ensured that the specimen is a segment of the upper ureter and did not incorporate any part of the renal pelvis. Upper and lower segments (3cm) taken just distal to the renal pelvis and proximal to the VUJ were used. This dissection method described ensured consistency and practicality of obtaining specimens. All segments were dissected gently to preserve the morphological and the physiological integrity of the tissue.

The stone was introduced into the ureteric segment, after control recordings had been made, at the top end using surgical forceps. Maximum care was taken when introducing the stone to prevent ureteric irritation or an effect on contractility. After a 15-30 minute period of recovery recordings were re-commenced, In control experiments, obstructions into the ureteric segments were not made but forceps was inserted at the top end to resemble the artificial procedure. In no cases was contractile function altered by this control procedure after the recovery period.

There is a possibility that aluminium salts that leach from the surface of the artificial obstruction might affect ureteric spontaneous contractions. Evidence from intestinal and gastric smooth muscle showed Al^{3+} affected that muscle motility but at high (millimolar) concentrations that would not have occurred by leaching from the surface of an aluminium foil ball (Nasu & Suzuki, 1998).

2.2.5 Data presentation and analysis

The amplitude of contractions was measured as the force generated by the tissue in grams, and the frequency of contraction was measured as the number of contractile events per 5 minutes.

Group data are presented as mean values ± SEM, and *n* refers to the number of preparations from which data were gathered.

Differences between data sets was tested by ANOVA with Tukey post hoc tests. The null hypothesis was rejected at $p<0.05$.

2.3 Results

Multiple specimens from each segment were investigated as shown in table 1. The pelvicalyceal system, renal pelvis and the upper ureter have demonstrated basal spontaneous activity in 100% of the specimens. The middle and lower ureteric segments demonstrated spontaneous activity in 67% and 65% respectively.

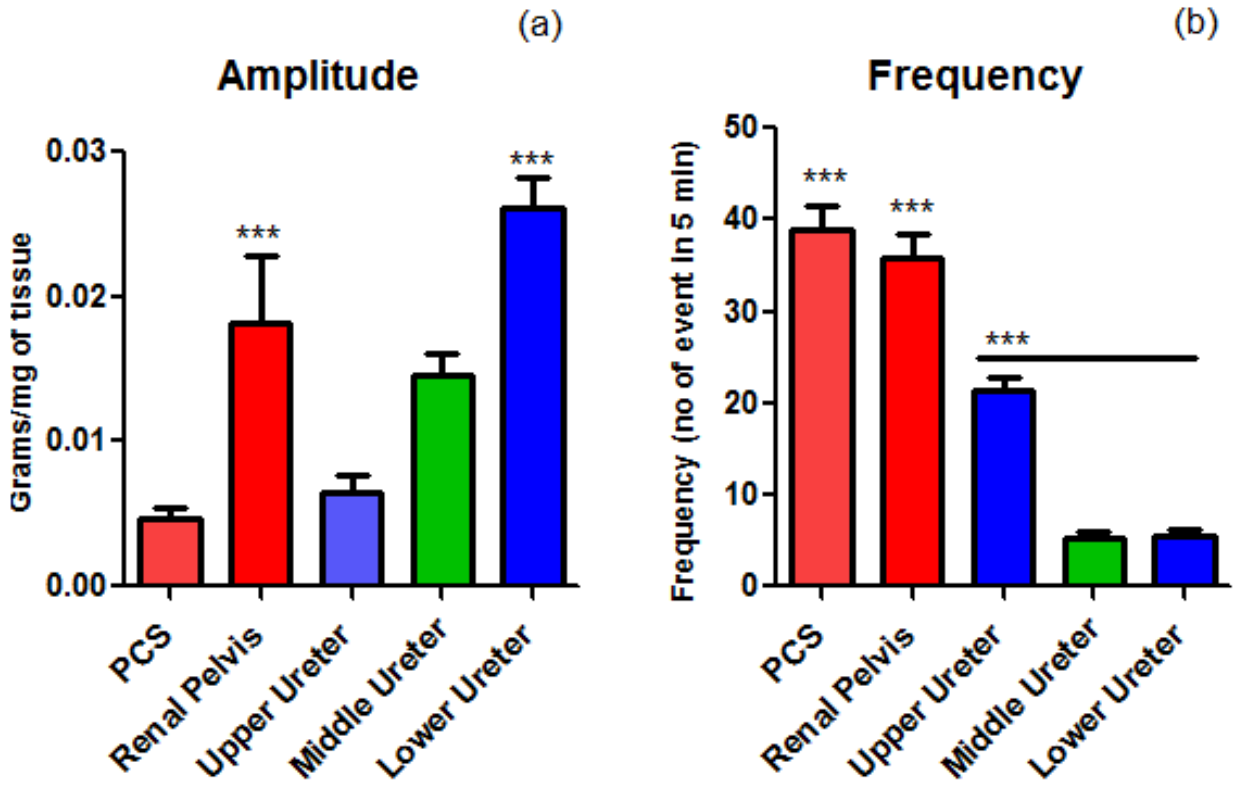
Also, in order to ascertain the average length of the pig ureter, the ureteric length was measured in 8 pigs, and the measurement of the mean length have been consistent with an average length of 25±3 cm.

Segment	Number of segments	Spontaneous activity
Pelvicalyceal system	32	100%
Renal Pelvis	26	100%
Upper ureter	38	100%
Middle ureter	18	67%
Lower ureter	26	65%

Chapter 2 - Table 1 - The number of ureteric segments of each type investigated and the percentage of basal spontaneous activity that each segment demonstrated.

Segment	Amplitude g/mg tissue	Frequency
Pelvicalyceal system	0.0046±0.0009	38.9±2.71 *(p<0.001)*
Renal Pelvis	0.0182±0.0046	35.9±2.51 *(p<0.001)*
Upper ureter	0.0065±0.0012	21.3±1.56 *(p<0.001)*
Middle ureter	0.0147±0.0015	5.3±0.71
Lower ureter	0.0262±0.0021 *(p<0.001)*	5.5±0.63

Chapter 2 - Table 2 - The amplitude and frequency of contraction of each segment. The amplitude of contraction presented as gram per milligram of tissue and frequency is presented as the number of events in 5 minutes.



Chapter 2 – Figure 2.

Fig.2 (a) - Bar chart demonstrating the amplitude of contraction of all the ureteric segments. The amplitude of contraction is represented in grams per milligrams of tissue. The stars sign (***) above the chart indicates a statistically significant result (*P<0.001*). The renal pelvis and the lower ureter both show a significantly higher amplitude of contraction.

Fig.2 (b) - Bar chart demonstrating the frequency of contraction of all the ureteric segments. The frequency of contraction is represented as the number of events in 5 minutes. The stars sign (***) above the chart indicates a statistically significant result (*P<0.001*). The pelvicalyceal system and the renal pelvis show a significantly higher frequency of contraction than the upper, middle and lower ureteric segments. The upper ureter demonstrates higher frequency than the middle and lower ureteric segments.

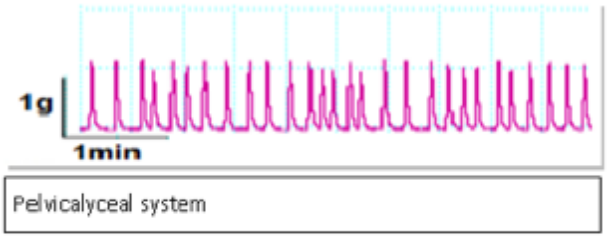


Fig3a

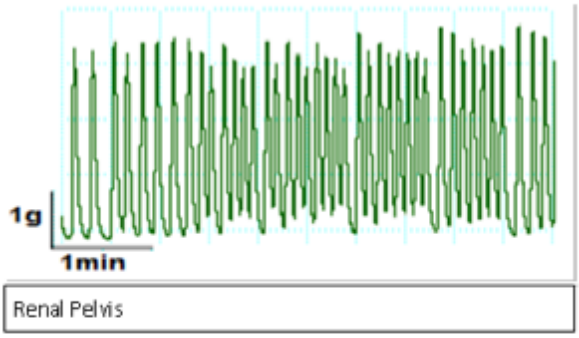


Fig3b

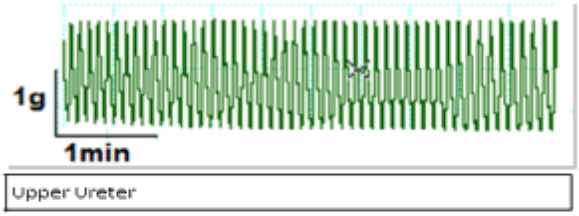


Fig3c

Fig3d

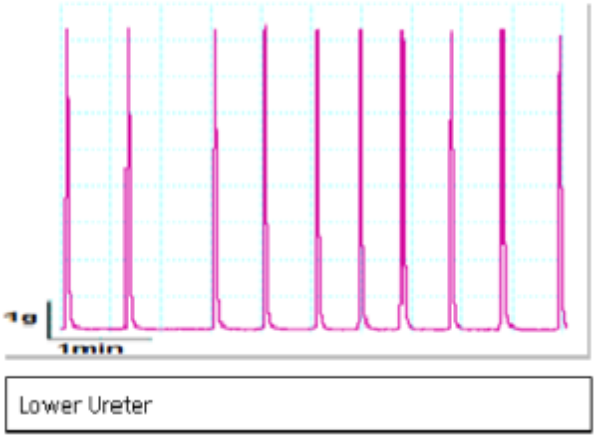


Fig3e

Chapter 2 - Fig 3 - The difference between the contractile pattern of all the segments of the upper urinary tract.
Fig 3a- pelvicalyceal system, Fig3b-renal pelvis, Fig3c-upper ureter, Fig3d- middle ureter

The Pelvicalyceal system - PCS

All the segments from the pelvicalyceal system demonstrated basal spontaneous activity. This segment had the lowest amplitude (0.0046±0.0009) of contraction but significantly ($p<0.001$) higher frequency (38.9±2.71) than the upper, middle and lower ureteric segments. Fig (2)

The Renal Pelvis - RP

The renal pelvis also demonstrated basal spontaneous activity in all segments, with significantly ($p<0.001$) higher amplitude (0.0182±0.0046) of contraction than the pelvicalyceal system (0.0046±0.0009), upper (0.0065±0.0012) and middle (0.0147±0.0015) ureteric segments but lower than (0.0262±0.0021- *$p<0.001$*) the lower ureteric segment . It also demonstrated lower contractile frequency (35.9±2.51) than the segments of the pelvicalyceal system but significantly($p<0.001$) higher contractile frequency than the upper, middle and lower ureteric segments – table 2.

The upper ureter - UU

The amplitude of the spontaneous activity in the UU segments (0.0065±0.0012) was lower than the renal pelvis, middle and lower ureteric segments – table 2. The contractile amplitude in the upper ureter was slightly higher than the pelvicalyceal system. The frequency (21.3±1.56) of contraction in the upper ureter was lower than the pelvicalyceal system and the renal pelvis but significantly higher ($p<0.001$) than the Middle (5.3±0.71) and lower (5.5±0.63) ureteric segments.

The Middle ureter - MU

The middle ureteric segments demonstrated basal spontaneous activity in 12/18 strips (67%). The amplitude (0.0147±0.0015) of contraction in the middle ureter was higher than the upper

ureter (0.0065±0.0012) and the pelvicalyceal system (0.0046±0.0009) but lower than the renal pelvis and the lower ureteric segments – table 2.

The frequency (5.28±0.71) of contraction in the middle ureter was significantly ($p<0.001$) lower than the pelvicalyceal system, renal pelvis and the upper ureteric segments but comparable to the segments from the lower ureter.

The Lower ureter - LU

In the lower ureteric segments, 17/26 (65%) of the strips demonstrated basal spontaneous activity. The lower ureteric segment had significantly ($p<0.001$) higher amplitude (0.0262±0.0021) than the pelvicalyceal system, renal pelvis, upper and middle ureter but lower frequency (5.5±0.63) than the pelvicalyceal system (38.9±2.71 *($p<0.001$)*, the renal pelvis and the upper ureteric segments. The lower ureter contractile frequency was comparable with the segments from the middle ureter (5.3±0.71) - table 2.

2.4 Discussion

Spontaneous ureteric contractions play an important role in the propulsion of urine from the kidney to the bladder and is dependent on the pacemaker activity that has been reported to originate from the proximal renal pelvis, upper, middle and lower part of the human ureter (Metzger et al. 2004). In pathological conditions such as the passage of kidney stones through the ureter, spontaneous activity plays an active role in the expulsion of ureteric stones (Nuss et al. 2005). Also, in the case of ureteric denervation after kidney transplantation, spontaneous contractions maintain ureteric peristalsis and the propulsion of urine (P Santicioli & Maggi, 1998).

In our current investigation of the spontaneous activity in the pelvicalyceal system, renal pelvis, upper, middle and lower ureteric segments; the porcine upper urinary tract demonstrated spontaneous activity in all the segments.

This study shows a progression of frequency and force characteristics which transition along the length of the upper urinary tract i.e. from the pelvicalyceal system to the lower ureter. The rhythmic pattern of contraction in the pelvicalyceal system with high frequency demonstrates the possibility of this segment initiating the peristaltic wave. This pyelo-ureteric autorythmicity has been demonstrated by (Lang et al. 2006; Gosling & Dixon 1978). The accumulation of urine in the renal pelvis along with receiving the peristaltic wave from the pelvicalyceal system can explain the higher amplitude of contraction in this segment in comparison with the upper ureter; the renal pelvis contracts with higher amplitude in order to overcome the pelviureteric junction and propel the urine forward to the upper ureter, this finding and interpretation was also described by Morita et al. (1981).

The amplitude of contraction gradually increases from the upper ureteric segment to the middle and then the lower ureter; this increase in contractile amplitude of the smooth muscles can be due to an intrinsic property of the lower ureteric segment. Our study has shown that the pelvicalyceal system had significantly higher frequency of contraction in comparison with the other segments. The frequency of contraction gradually decreases from the pelvicalyceal system onwards. The renal pelvis and the upper ureter demonstrated significantly higher frequency of contraction in comparison with the middle and lower ureteric segments. Fig (2) demonstrates the amplitude and frequency of contractions in all segments, and it is possible to comment on the inverse relationship between both the frequency and amplitude of contraction. As the frequency increases in a segment, the amplitude decreases, this is observed in all segments except the renal pelvis. The segments

that have demonstrated higher frequency like the pelvicalyceal system, renal pelvis and upper ureter, do not need to have a high amplitude as the urine volume is not large and frequency seem to be of higher importance to continue the propelling action and also to transmit the electrical impulse to the rest of the ureter in order to maintain the peristaltic wave. There is a gradual decrease in ureteric diameter from the upper to the lower segments and as the urine bolus traverses the ureter; although the volume of urine could be constant, the decrease in diameter may cause the lower segments may require a higher amplitude to propel the bolus of urine forward towards the bladder.

The renal pelvis seems to play a pivotal role in the conduction of the peristaltic wave and in the mechanics of moving the urine bolus through the pelviureteric junction and subsequently along the ureter. It is the only segment that demonstrated high frequency and amplitude of contraction. The significance of this finding has been discussed earlier in light of the findings by Morita et al. (1981)

The contractile behavior of the ureteric segments seems to be demonstrating a pattern of contraction based on amplitude and frequency; this contractile pattern seems to be coordinated along all segments as the urine bolus traverses from the kidney to the bladder. Perhaps through these behavioral patterns, an analysis of the individual role of each segment can be proposed. The pelvicalyceal system seems to have the highest frequency but with low amplitude, the role of this segment can be the transfer of small volumes of urine from the calyces and to initiate peristalsis. The upper ureter receives the urine bolus from the renal pelvis after passage through the pelviureteric junction; this segment has high frequency of contraction comparable with the renal pelvis but a much lower amplitude. As the urine bolus has passed through the PUJ, there is no need for high amplitude muscle contraction and only the peristaltic action potential is required to pass on to the lower segments. The upper ureter

seems to have a more significant function in conducting the action potential of contraction and just augmenting the bolus of urine to pass forward into the middle ureter. The middle ureteric segment has low frequency and high amplitude of contraction; this is also true for the lower ureter. As urine passes through these segments, there is a gradual increase in the amplitude of contraction as the volume of urine may exert pressure on the ureteric wall. The middle ureter seems to add an augmentation to the amplitude of contraction to the lower ureter along with simply traversing the urine bolus. The lower ureteric segment interestingly has the highest amplitude of contraction yet with a low frequency. As described for the renal pelvis and the PUJ, the lower ureter has to also contract against a physiological junction; the vesicoureteric junction. Both the bolus of urine and the contractile wave generated in the upper segments and then the middle ureter, seem to conglomerate in creating this significant contraction.

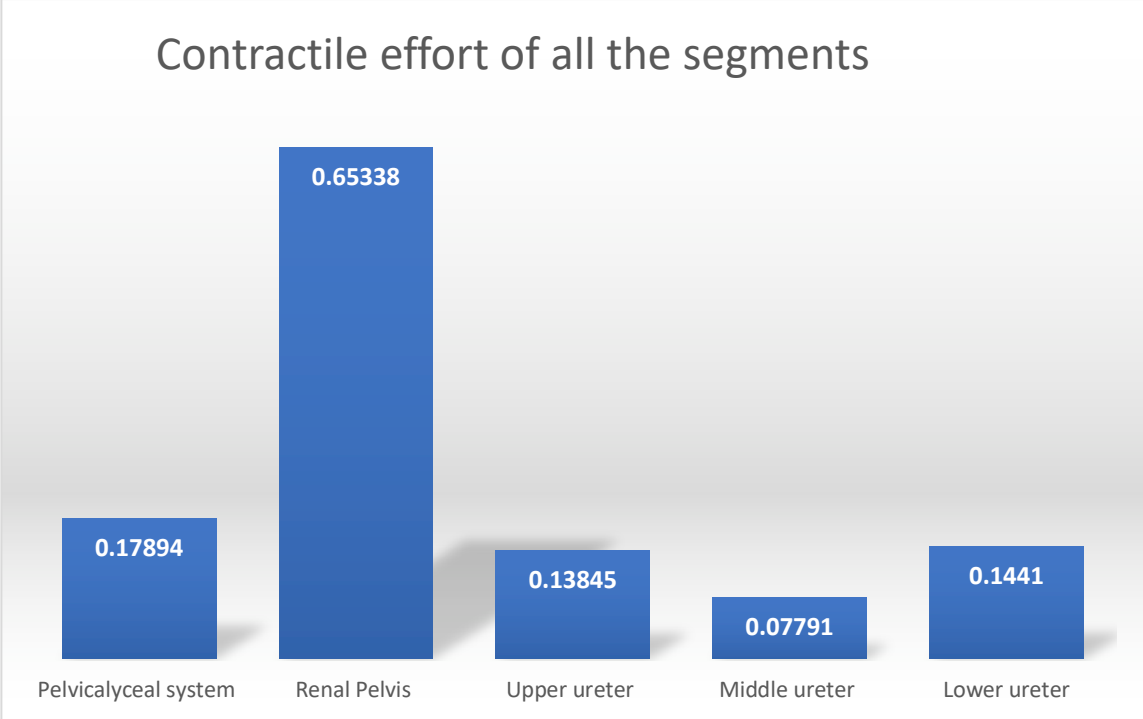
The difference in the contractile behaviour of the pelvicalyceal system, renal pelvis, upper, middle and lower ureter may indicate that each segment plays a different role in mediating the peristaltic wave. Understanding the difference in the basal contractile behaviour of the upper urinary tract may provide insight into the development of a targeted approach in the management of ureteric conditions.

To further elucidate the relationship between the amplitude and frequency of contraction measured as the contractile effort (amplitude*frequency). There seems to be a constant relationship between the effort exerted by the ureter and the contractile behaviour of each segment and the effort required to propel the bolus of urine. The PCS, UU, MU and LU segments demonstrated a balanced of contractile effort at each of those segments that are consistent with the flow dynamics of urine through each segment and the frequency and amplitude demonstrated above. The RP is the segment with the highest contractile effort that

further demonstrates the importance of the renal pelvis as the ureteric segment that may be playing a significant role in ureteric peristalsis and conditions like PUJ obstruction and renal pelvis obstructing stones should be carefully addressed.

Segment	Amplitude	Frequency	Contractile effort
Pelvicalyceal system	0.0046	38.9	0.17894
Renal Pelvis	0.0182	35.9	0.65338
Upper ureter	0.0065	21.3	0.13845
Middle ureter	0.0147	5.3	0.07791
Lower ureter	0.0262	5.5	0.1441

Chapter 2 – Table 3: Contractile effort of all segments.



Chapter 2 – Figure 4: Contractile effort of all segments.

Chapter 3

The effect of cholinergic receptor modulators on upper, middle and lower ureteric segments.

3.1 Introduction:

Electron microscopy has demonstrated that adrenergic and cholinergic nerve terminals are in close proximity in the human ureter (Schulman, 1975). Ganglion cells in the ureter were described by Langley et al. as early as 1894. The two divisions of the autonomic nervous system, sympathetic and parasympathetic, play an active role in modulation of ureteric activity by affecting the frequency and amplitude of peristalsis and hence the volume of urine transported (Morita, Wada, Suzuki, *et al.*, 1987). The upper ureteric segment receives sympathetic innervation from spinal cord segments T10-12 and parasympathetic innervation from the dorsal efferent nucleus of the vagus nerve (Langley & Anderson 1896; Mitchell 1935; Kuntz et al. 1957). The sympathetic innervation of the lower segment of the ureter is from the L1,2, and the parasympathetic component from S2,3 (Langley and Anderson, 1894; Swenson *et al.*, 1952).

Cholinergic agonists stimulate ureteric contractions *in vitro* in numerous animal preparations including cats, horses, dogs and pigs (Deane, 1967; Yano *et al.*, 1984; Theobald, 1986; Prieto *et al.*, 1994). Moreover, effects have also been shown *in vivo* in anaesthetised animals (Rose and Gillenwater, 1974; Theobald, 1986; Latifpour *et al.*, 1989; Prieto *et al.*, 1994). α-adrenoceptors modulate ureteric contractions and relaxation is mediated by β-adrenoceptors (Deane, 1967). The effect of α-adrenoceptors has been widely studied in the ureter with emphasis on the role of α-adrenoceptor blockers in management of ureteric stones. However,

the role of cholinergic receptors modulators requires further investigation, especially their role in modulating ureteric contraction and their potential role in the management of ureteric stones.

The density of muscarinic receptors and cholinergic nerves varies along the ureter; smaller in the proximal ureteric segments and denser in the lower and intravesical segment of the ureter (Del Tacca, 1978; Hernández, García-Sacristán & Orensanz, 1995). Conventional neuromuscular junctions are absent on ureteric smooth muscle, hence activation depends on a diffuse release of a neurotransmitter from nerves and subsequent spread of electrical activity by conduction between ureteric myocytes (Uehara & Burnstock, 1970). Moreover, the ureter has the ability to spontaneously contract and exhibit peristalsis even if it has been denervated; demonstrated by *in vitro* experiments and in humans after kidney transplantation (O'Conor, Dawson-Edwards 1959).

Five muscarinic receptor subtypes (M1–M5) have been demonstrated in the human ureter with immunohistochemistry studies; however, RT-PCR analysis identified only M2, M3- and M5-receptor subtypes (Sakamoto, Suri & Rajasekaran, 2006c). Acetylcholine is a cholinergic agonist and functions both as a neurotransmitter at preganglionic parasympathetic neuroeffector junctions (nicotinic sites) and postganglionic parasympathetic neuroeffector junctions (muscarinic sites). The presence of M2 receptors in the porcine intravesical ureter and M1, M3 and/or M4 subtypes seems to be responsible for the cholinergic innervation of the ureter in the porcine model. Stimulation of muscarinic receptors by carbachol produces contraction of the pig isolated intravesical ureter.

Hernandez et al. demonstrated that both M1 and M3 muscarinic receptor subtypes mediate the increases in the basal tone. At the same time, M2-receptors are involved in the increase

in the frequency of phasic contractions in the isolated porcine intravesical ureter in response to cholinoceptor agonists. (Hernández *et al.*, 1993; Hernández, García-Sacristán & Orensanz, 1995). However, Roshani et al 2003 showed that smooth muscle activity in the mid and the distal pig ureter was not modulated by muscarinic receptors (Roshani *et al.*, 2003). However, in the latter study insertion of the ureteric catheter, as part of the experimental procedure, caused reduction of peristalsis, as subsequently described elsewhere (Venkatesh *et al.*, 2005). Thus, although the influence of the parasympathetic nervous system to modulate ureteric contractility has been described the effect on different ureteric segments is less clear, although the different density of innervation along the ureter may suggest regional variation (Paolo Santicioli & Maggi, 1998).

3.2 Hypothesis

Cholinergic receptor activation has varying effects on different segments of the ureter.

Aim: To investigate the effect of carbachol, a muscarinic and nicotinic receptor agonist and atropine, a muscarinic receptor antagonist on the contractile activity of different segments of the pig ureter.

Objectives: In vitro experiments with pig ureter measured the amplitude and frequency of spontaneous contractions in the upper, middle and lower segments of the pig ureter.

3.3 Methods:

The methods for this chapter are explained in chapter 2.

3.4 Results:

The ureteric specimens were collected from different pigs and each ureteric segment was investigated in a separate experimental setup.

All the upper ureteric segments (n=23), demonstrated basal spontaneous activity (BSA) (100%). The middle (n=24) and lower ureteric segments (n=24) demonstrated lower percentage of spontaneous activity; (n=13) 54.2% and (n=17) 70.8% respectively. For the experiments comparing basal and carbachol stimulated spontaneous activity; (n=16) UU segments, (n=13) MU segments and (n=17) LU segments were investigated.

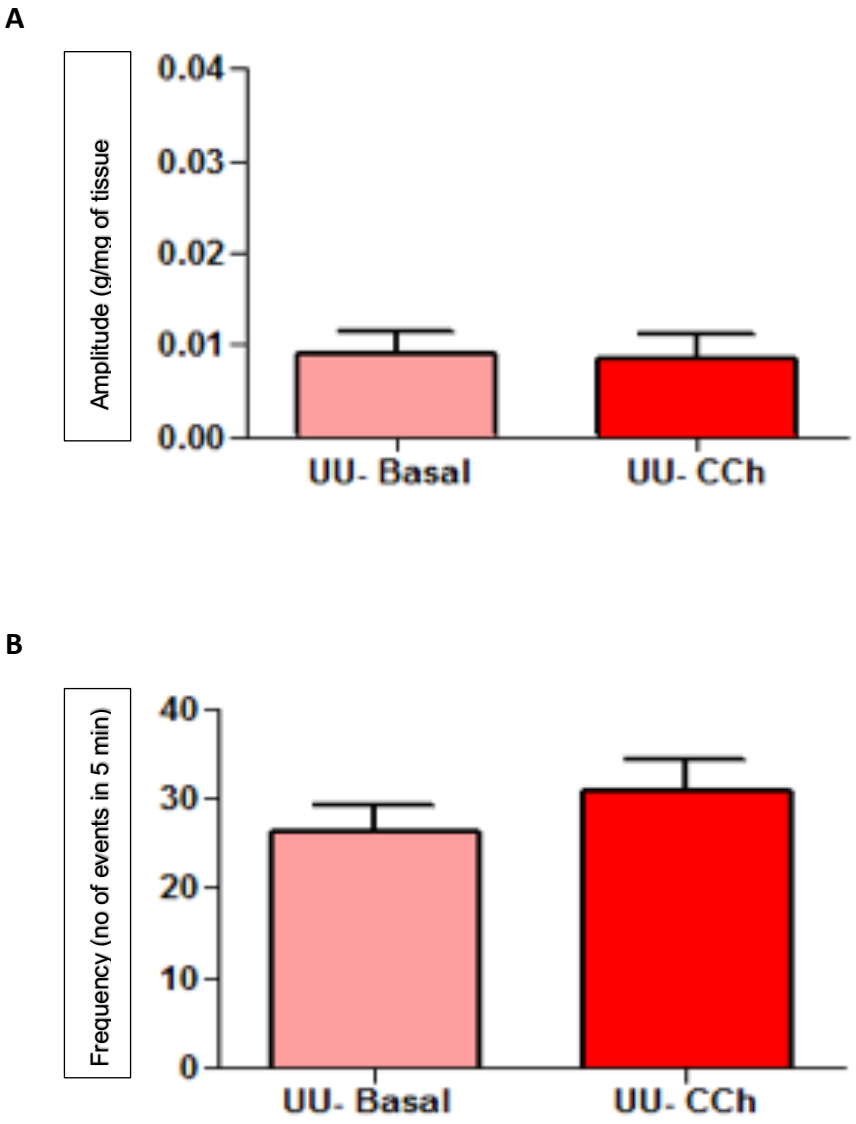
The amplitude of BSA in the lower ureter (0.02±0.004 g tension/mg tissue) was significantly *(P<0.001)* higher than the middle (0.013±0.002 g tension/mg tissue) and the upper ureteric segments (0.009±0.002 g tension/mg tissue). However, the frequency of BSA was significantly ($p<0.001$) higher in the upper ureter (26.38±2.77 events in 5min) versus the middle (4.92±0.92 events in 5 min) and lower ureteric segments (4.18±0.94 events in 5min).

The basal amplitude in the UU was the lowest in comparison with the MU and LU segments. The frequency of basal contractions in the UU segments were higher than the MU and LU segments. The MU and LU segments basal contractile frequency were comparable.

MU basal amplitude was not significantly different from UU segments (0.013±0.002 g/mg) but the frequency was significantly lower (4.92±0.92 per 5-min). LU basal amplitude was significantly greater than in the other two segments (0.02±0.004 g/mg) with a frequency similar to that in MU segments (4.18±0.94 per 5-min). Thus, all three segments showed different amplitude and frequency characteristics of spontaneous contraction.

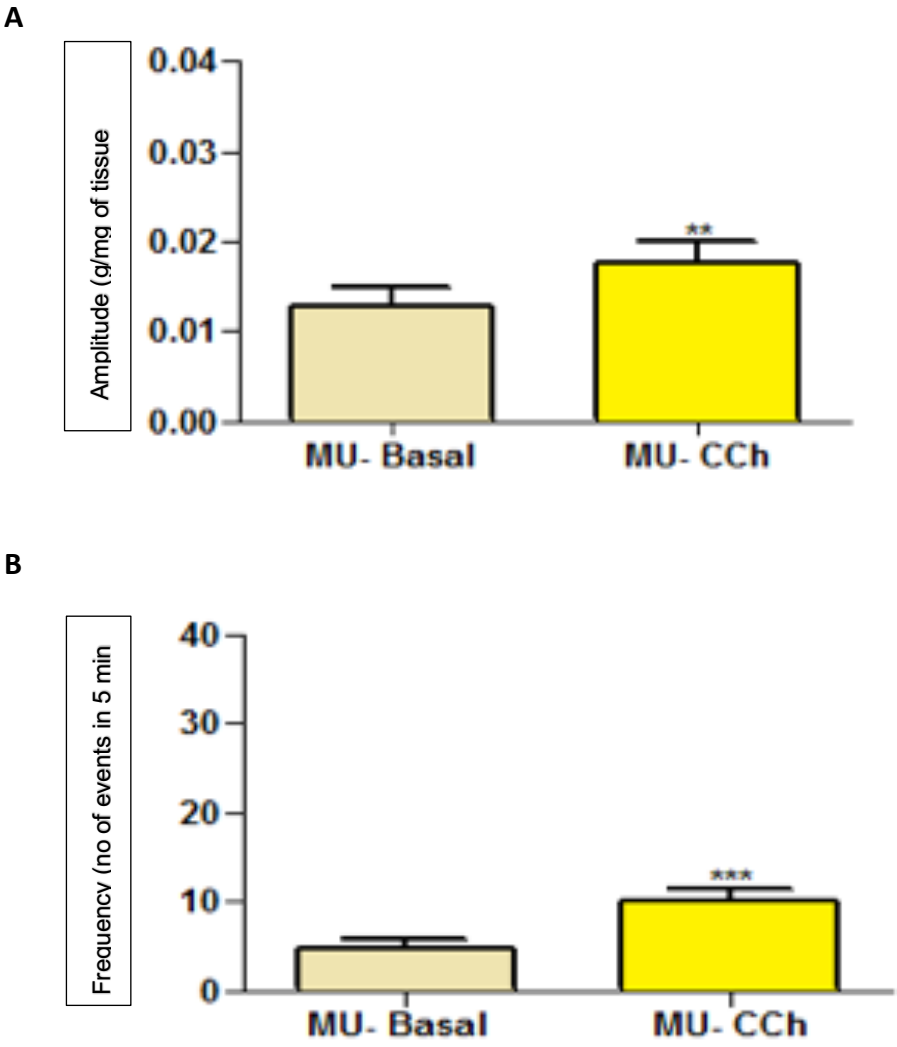
In the Carbachol stimulated segments; the drug had no impact on the amplitude and frequency of contraction in the UU segments. In the MU segments; Carbachol significantly increased the amplitude and frequency of contraction. The LU segments have also shown a significant response to Carbachol with significant rise in contractile amplitude and frequency.

Effect of carbachol on the upper ureter. Carbachol (10 µM) had no effect on the amplitude or frequency of spontaneous activity in upper ureteric segments (Fig 1).



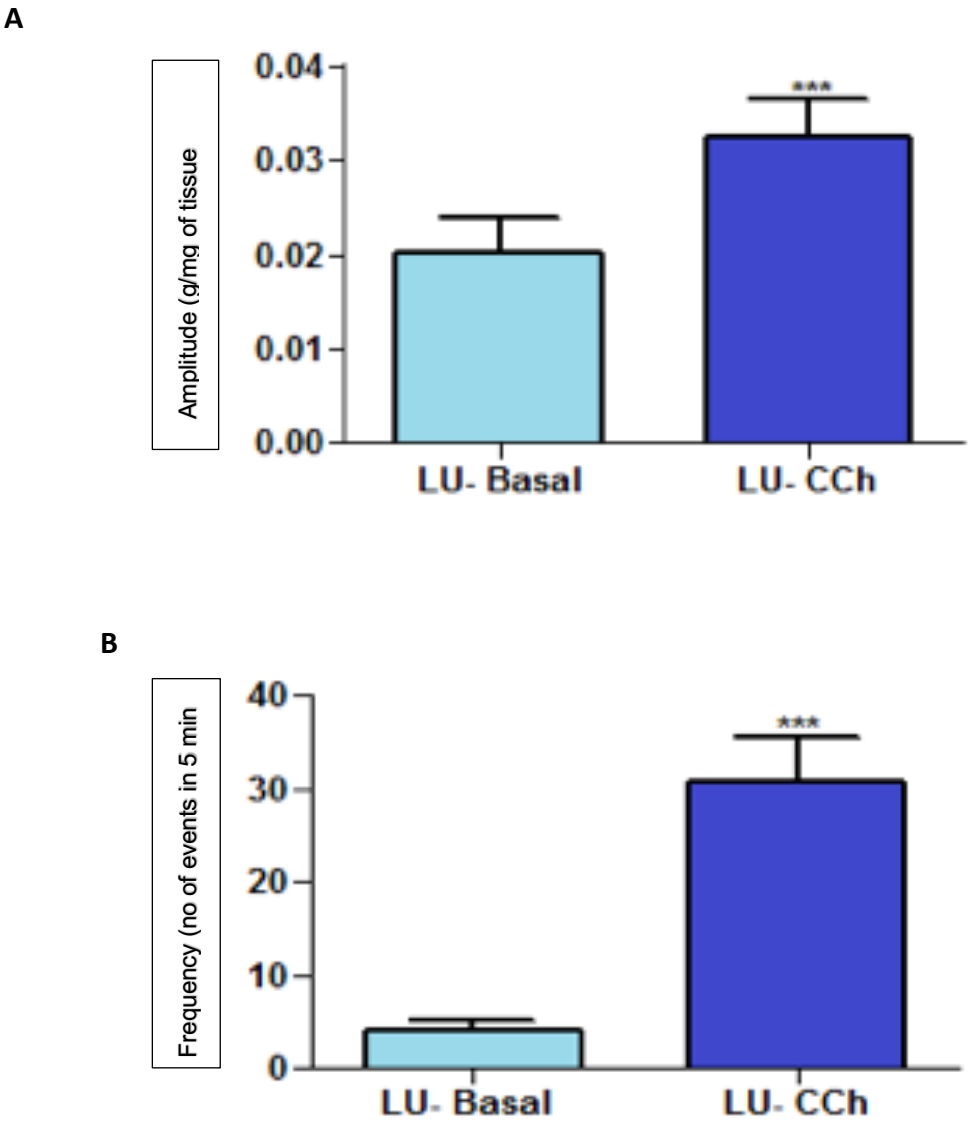
Chapter 3 – Fig .1. The effect of carbachol on upper ureter (UU) spontaneous contractions. A: contration amplitude. B: contraction frequency. Data mean±SEM (n=16).

Effect of carbachol on the middle ureter. Carbachol (10 µM) significantly increased both the amplitude and frequency of spontaneous activity in middle ureteric segments (Fig 2).



*Chapter 3 - Fig 2. The effect of carbachol on middle ureter (MU) spontaneous contractions. A: contraction amplitude. B: contraction frequency. Data mean±SEM (n=13),**p<0.01, ***p<0.001*

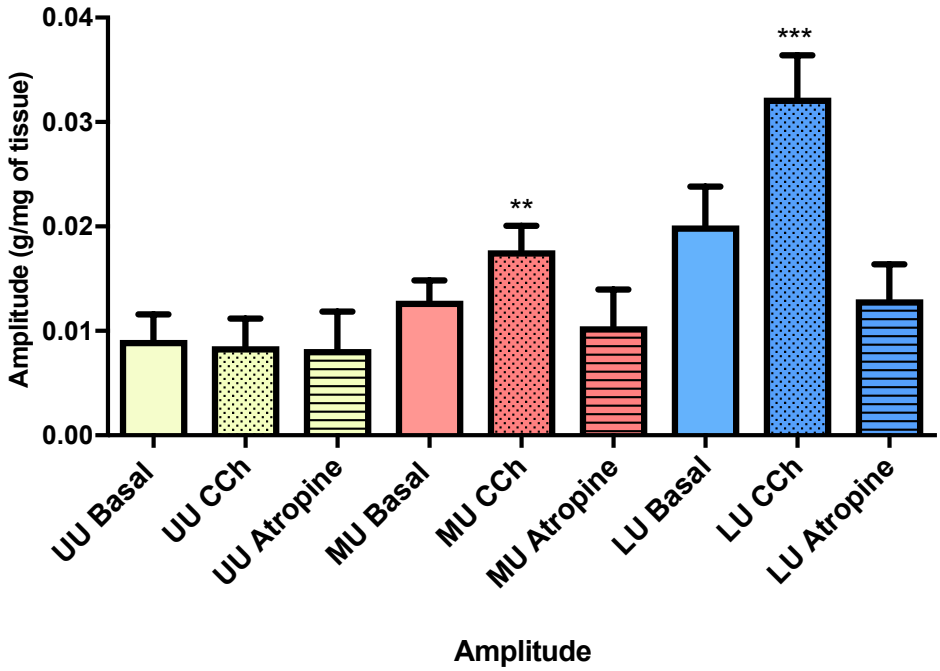
Effect of carbachol on the lower ureter. Carbachol (10 µM) significantly increased both the amplitude and frequency of spontaneous activity in lower ureteric segments (Fig.3). Proportionately the effect on frequency was particularly great, more so than on the middle ureter.



*Chapter 3 - Fig 3 The effect of carbachol on lower ureter (LU) spontaneous contractions. A: contraction amplitude. B: contraction frequency. Data mean±SEM (n=17), ***p<0.001*

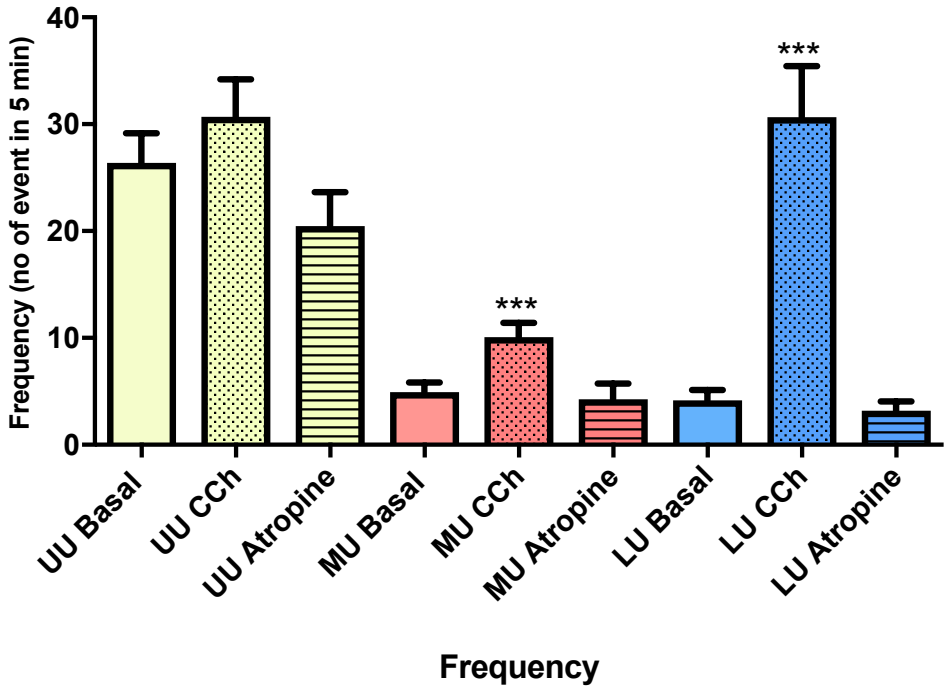
Atropine 1µM had no effect on the amplitude and frequency of contraction on the upper ureteric (n=12), middle (n=13), and lower ureteric segments (n=14).

A summary of the data is shown in Fig. 4 for amplitude (part A) and frequency (part B). The data shows basal activity values and any action of carbachol. Shown is the variation of amplitude in the three segments of the ureter, as well as augmentation of amplitude by carbachol in middle and lower ureter segments.



Chapter 3 – Fig 4. Summary of amplitude data in the upper, middle and lower ureteric segments. The effects of 10 µM carbachol and 1µM atropine added separately *are shown. Mean±SEM (see Figs 1-3 for n-values); **p<0.01; ***p<0.001*

Fig. 5 shows similar data for frequency, variation of the basal frequency and augmentation of contractile frequency by carbachol in the middle and lower segments; with no effect of atropine.



Chapter 3 - Fig 5. Summary of frequency data in the upper, middle and lower ureteric segments. The effects of 10 µM carbachol and 1µM atropine added separately *are shown. Mean±SEM (see Figs 1-3 for n-values); ***p<0.001*

3.5 Discussion:

This study demonstrated a difference between the basal and carbachol-dependent contractile behaviour of the upper, middle and lower segments of the pig ureter. The frequency of basal spontaneous activity was significantly higher in the upper ureter compared to the middle and lower ureteric segments. In contrast, the amplitude of basal activity was significantly higher in the lower segment compared to the upper and middle ureter.

These data corroborate a previous study whereby carbachol generated contractions if isolated pig ureter, but in this case no differentiation was made between different segments

(Hernández et al., 1995). The abolition of carbachol-mediated responses by atropine indicates that it acts on muscarinic receptors. Tomiyama et al. (2003) demonstrated that carbachol altered ureteric contraction by increasing the contractile frequency in isolated canine ureter, possibly mediated by the M3 receptor. Morita et al. (1987) showed in dog ureter that atropine alone had no significant effect on contractile function, indicating little basal acetylcholine release. However, a study in anesthetised dogs study (Tomiyama et al., 2004) showed cholinergic receptor stimulation by carbachol had a suppressive effect on ureteric pressure and peristalsis in obstructed ureters.

The nature of the muscarinic receptor subtype is of interest and all five (M1-M5) have been demonstrated in ureter (Sakamoto, Suri & Rajasekaran, 2006). Detrusor smooth muscle contains M2 and M3 receptors, with M3 as the functionally relevant subtype (Uchiyama & Chess-Williams, 2004), whereas M1 receptors are largely found on nerves. The M4 receptor is primarily found in the CNS (Weiner, Levey & Brann, 1990)], its distribution largely overlapping with that of M1 and M3 subtypes. M4 receptors are inhibitory auto-receptors for acetylcholine (activation will inhibit acetylcholine release) and this has been observed in human detrusor. The role of the M5 receptor is unclear. A further study is therefore required to determine the effect of different muscarinic receptor subtype inhibitors to shed light on the prominent subtype that regulates ureteric contraction.

This study has shown a complex relationship between contractile frequency and amplitude in modulating ureteric contractile function. As carbachol had no discernible effect on upper ureteric segments, but both agents affected the frequency and amplitude of the middle and

lower ureteric contractility this implies that parasympathetic modulation of activity is primarily in the middle and distal regions of the ureter.

A comparison of the contractile properties of the upper, middle and lower segments of the pig ureter is novel. This suggests that cholinergic modulation does not primarily modulate initiation of contractile activity but modulates downstream peristalsis. Whether such a differentiation of action in the human ureter is present would be interesting to evaluate. A first step would be to carry out a segmental analysis of muscarinic receptor distribution by immunohistochemistry

Chapter 4

The effect of obstruction on ureteric contractility; comparison between the upper and lower ureteric segments.

4.1 Introduction

Ureteric obstruction can have a deleterious effect on the kidney and may present in various ways. The notion of obstruction implies that there is a discrepancy or imbalance between urine production and drainage from the kidney to the bladder. Ureteric pathology or obstruction can affect both the paediatric and adult age groups. The degree of renal injury depends on the severity of obstruction, baseline renal function, whether the obstruction is acute or chronic and the presence of other mitigating factors such as urinary tract infection or anatomical abnormalities of the urinary tract. The impact of obstruction on renal function can be significant with resultant physiologic and histologic changes that may result in permanent renal damage.

4.1.1 Clinical presentation of ureteric obstruction

The acute presentation of renal colic usually occurs with the passage of a kidney stone. The classic symptoms are flank pain accompanied by nausea and vomiting due to distension of renal pelvis and visceral response. The pain radiates to the lower abdomen, testicles, or labia. In cases of chronic obstruction, the patient is relatively asymptomatic or complains of occasional discomfort. The obstruction to urinary flow can occur anywhere along the urinary tract, which can be from the kidney to the urethral meatus. The upper urinary tract, which is

the kidney and the ureter and the lower urinary tract which is the bladder and urethra up to the level of the urethral meatus. This division allows for the delineation and description of the aetiology and impact of obstruction. In the upper urinary tract, three specific regions are more prone to obstruction; these include the pelviureteric junction, the crossing of the ureter over the area of the common iliac vessels- the pelvic brim and the ureterovesical junction. The area of ureteric narrowing at the pelvic brim is unique due to two reasons; which is the extrinsic compression by the iliac vessels and the anterior angulation of the ureter as it crosses the vessels to enter the pelvis. There is no actual intrinsic change in the ureteric calibre in this part of the ureter (Wein *et al.*, 2016).

Obstruction can either be due to intrinsic or extrinsic aetiologies which can induce compressive or restrictive forces on the urinary tract. Calculi, blood clots, sloughed papilla and tumours can cause intraluminal obstruction. The acute onset of intraluminal obstruction may lead to renal colic with flank pain, haematuria, nausea, vomiting, and fever. Another cause of intraluminal obstruction which can affect the renal function over time is ureteric strictures which can be caused by stone disease, malignancy, or iatrogenic causes such as ureteroscopy. Ureteric strictures tend to develop over a period and may cause a chronic obstruction which may ultimately lead to renal atrophy. In females, the distal ureter crosses the posterior aspect of the pelvic blood vessels and the broad ligament. The ureters can become externally compressed by pelvic tumours such as advanced gynaecologic malignancies. Also in women, prolapse of the pelvic structures such as the uterus can lead to urinary outflow obstruction. In pregnancy, the gravid uterus can lead to ureteric obstruction. (Carey *et al.*, 1989) In males, benign prostatic enlargement and urethral strictures can affect the urine flow and can cause urinary outflow obstruction (Lepor, 2004).

There are other less common causes of ureteric obstruction such as inhibition of ureteric peristalsis, or an aberrant lower pole crossing artery that is oriented anterior to the ureter and can exert pressure at the level of the pelviureteric junction or the upper ureter and cause obstruction. Other vascular causes include abdominal aortic aneurysms and common iliac artery aneurysms which can externally compress the ureter. Vascular grafts placed for aneurysm repair can cause hydronephrosis in up to 10-20% of patients from mechanical obstruction of the ureter, they may spontaneously resolve Retroperitoneal fibrosis can cause constriction around the ureter and prevent peristalsis of one or both ureters, in 8% to 10% of cases there is a coexistent malignancy. Retrocaval ureter which is an embryologic anomaly develops in utero due to the persistence of the posterior sub-cardinal vein which may lead to obstruction as the ureter passes posterior to the inferior vena cava, this occurs on the right side with male predominance. Ureteric obstruction becomes symptomatic in the third to fourth decade of life (Daune et al. 1991).

In the paediatric population, the leading causes of ureteric obstruction are a pelviureteric junction or vesicoureteric junction obstruction, ectopic ureter, ureterocele, and megaureter (Shalaby-Rana et al. 1997).

Unilateral ureteric obstruction

In unilateral ureteric obstruction, there are three main phases; in phase one there is a rise in ureteric pressure and renal blood flow which lasts for one to two hours. The Vasodilation of the afferent arterioles increases RBF which offsets the fall in GFR. The tubule-glomerular feedback mechanism, prostaglandin E2 and nitric oxide mediate this response (Rajasekaran, Maxvold and Bunchman, 2011). The decline in glomerular filtration rate is gradual, and the

decrease occurs over few hours. There is a drop in GFR by 50% in four hours, 75% in twelve hours and a further drop by 95% within twenty-four hours (Pickering & Endre, 2014).

In the second phase, there is a persistent rise in ureteric pressure as this phase lasts for three to four hours. During this period, the renal blood flow begins to decrease as vasoconstrictors such as angiotensin II and thromboxane A2 play a vital role during this phase. In the experimental model, the administration of angiotensin-converting enzyme (ACE) inhibitor will mitigate the decline in GFR and renal blood flow, which provides evidence that angiotensin II plays a vital role in this process (Rajasekaran, Maxvold & Bunchman, 2011).

The third phase begins after five hours of the obstruction. The ureteric pressure and the renal blood flow both start to decline at this stage as the afferent arteriolar resistance decreases. During this phase, the renal blood flow perfuses mainly the medullary regions as the blood shifts from the outer cortex. This shift in blood flow results in a lack of perfusion to a large number of glomeruli, and further reducing the GFR 1. The decline in renal blood flow over time becomes significant as it decreases to 70% at 24 hours, 50% at 72 hours, 20% at two weeks and 12% at eight weeks (Pickering & Endre, 2014).

Bilateral ureteric obstruction

During the early phase of bilateral ureteric obstruction, the renal blood flow may slightly increase for approximately 90 minutes as nitric oxide is thought to mediate this mechanism. The renal blood flow then remarkably declines and is much more significant than in unilateral obstruction, and there is a significant decrease in medullary blood flow in bilateral obstruction. The vasoconstrictors thromboxane A2, endothelin and angiotensin II mediate this phase. Ureteric pressure is much higher in the case of bilateral obstruction and remains elevated for at least 24 hours. The hypothesis behind this notion is that there is an

accumulation of vasoactive substances such as atrial natriuretic peptide (ANP) that do not usually accumulate in unilateral obstruction as the contralateral kidney would excrete these substances (Klahr & Morrissey 2002).

4.1.2 Role of ANP in ureteric obstruction:

Atrial natriuretic peptide (ANP) accumulates in bilateral ureteric obstruction (Purkerson *et al.*, 1989). Bilateral ureteric obstruction leads to an increase in the intravascular fluid volume that manifests in an increase in pulmonary capillary wedge pressure and body weight; these changes stimulate the secretion of ANP. ANP increases afferent arteriolar dilation and efferent arteriolar vasoconstriction, thus increasing the glomerular capillary pressure. It also decreases the sensitivity of tubule-glomerular feedback, inhibits the release of renin, and increases glomerular ultrafiltration coefficient (Fried, 1987; Cogan, 1990).

4.1.3 Cellular and Molecular Changes

The effect of ureteric obstruction on the kidney starts to have an impact on the renal tubules as these cells start to atrophy and die by apoptosis. The glomerular cells appear to be resistant to the deleterious manifestations of obstruction. Caspases which are cysteine aspartate-specific proteinases mediates the apoptosis of tubular cells. These caspases generate significant inflammatory responses and the release of cytokines. An essential cytotoxic cytokine that plays a significant role in the apoptotic process is TNF-α (Ortiz, 2000).

Continued urinary tract obstruction leads to the development of tubulointerstitial fibrosis and interstitial inflammation which results eventually in permanent changes to the structure of the kidney. In addition to TNF-α, other essential cytokines and growth factors that a play a

role in these changes are TGF-β, angiotensin II and NFκB. Obstruction also causes accumulation of extracellular matrix which further contributes to fibrosis (Schnaper, 2017).

4.1.4 Initiation of fibrosis due to cellular and molecular changes

The obstruction of the urinary tract eventually commences progressive and potentially permanent changes in the structure of the kidney, which including the development of tubulointerstitial fibrosis, interstitial inflammation, apoptosis and tubular atrophy.

Various cytokines and growth factors play a role in the inflammatory changes that occur in the kidney; the most notable is the transforming growth factor-beta TGF-β, angiotensin II, NFκB, and TNF-α. Some are produced directly from the renal tubular and interstitial cells and some cytokines are produced by infiltrating macrophages.

The extracellular matrix is synthesised and deposited at a rate higher than its degradation and consequently leads to the development of tubulointerstitial fibrosis. Also, the collapse of the parenchymal volume leads to an increase in the matrix as nephrons damaged (Hewitson, 2009).

Obstruction increases the synthesis of metalloproteinase inhibitors. Matrix metalloproteinases and collagenase are a group of enzymes that cleave and degrade the collagenous and non-collagenous components of the extracellular matrix. Hence it reduces metalloproteinase activity and results in the accumulation of extracellular matrix. The infiltrating macrophages stimulate the synthesis of TGF-β, which is a growth factor that increases tissue inhibitor of metalloproteinases (TIMP) production, thus reducing collagen turnover. Macrophages additionally produce other cytokines and growth factors, such as interleukin 2, interleukin 6, fibroblast growth factor, and platelet-derived growth factor

(PDGF), that seem to contribute to the process of inflammation and fibrosis (Sutaria *et al.*, 1998).

The activated type 1 TGF-β receptor subsequently phosphorylates SMAD which are mobile transcription factors. A heteromeric complex of SMAD proteins translocates to the nucleus, where it interacts with transcription factors to regulate gene transcription (Wamsley-Davis *et al.*, 2004) and stimulates tubulointerstitial fibrosis (Fukasawa *et al.*, 2004). The events that lead to fibrosis are thought to be initiated by the rise in angiotensin II levels; also, other profibrotic factors seem to play a significant role because inhibition of angiotensin synthesis by ACE inhibitors or antagonism of the AT1 receptors reduces but does not completely eliminate the fibrotic process (Kaneto, Morrissey & Klahr, 1993; Ishidoya *et al.*, 1995)

In essence, obstruction of normal urine outflow results in biochemical, immunologic, hemodynamic, and functional changes. It stimulates a cascade of events in which elevated levels of angiotensin II, cytokines, and growth factors lead to tubular cell apoptosis and cellular inflammation, increased net matrix formation, and tubulointerstitial fibrosis. Many of the mediators are intrinsic to the renal tubular cells, and others are provided by fibroblasts and by migrating macrophages.

4.1.5 Post-obstructive Diuresis.

The resolution of bilateral ureteric obstruction results in increased urine flow 3 to 10 fold; this physiologic post-obstructive diuresis helps to excrete the salts retained during obstruction. However, there is pathologic diuresis that may occur when the kidneys excrete water and electrolytes that is more than that retained during the obstruction (Basile, Anderson & Sutton, 2012).

During the period of significant polyuria, urine output of 200 mL/hr or higher may occur. Diuresis occurs mainly after relief of bilateral ureteric obstruction or obstruction of a solitary kidney, it rarely occurs when there is a healthy, contralateral kidney (Schlossberg & Vaughan, 1984).

Diuresis is typically a normal physiologic response to the volume expansion and solute accumulation that occurs during obstruction. Sodium, urea, and water are excreted, and diuresis subsides after solute, and fluid homeostasis is achieved (Loo *et al.*, 1988).

Though, a "pathologic" post-obstructive diuresis may ensue due to inappropriate renal handling of water or solutes or both. It is due to derangement of the medullary solute gradient and several altered signalling and transport pathways. The downregulation of sodium transporters with subsequent impaired sodium reabsorption in the thick ascending limb of the loop of Henle prevents maintenance of the medullary interstitial solute gradient, promoting a continued diuresis (Rokaw *et al.*, 1996).

The increased endogenous production and altered regulation of ANP induce saline diuresis (Soo Wan Kim *et al.*, 2001). Also, other natriuretic peptides, such as the Dendroaspis natriuretic peptide, may contribute to the process. Pathologic post obstructive diuresis is characterised by the inadequate response of the collecting duct to antidiuretic hormone (ADH); this is considered to be due to a downregulation of aquaporin water channels in this part of the nephron and perhaps in the proximal tubule, which has been shown in experimental models of unilateral and bilateral ureteric obstruction. (Nielsen & Agre, 1995; Gregersen *et al.*, 1996; S W Kim *et al.*, 2001).

Several concepts have been proposed for the rare occurrence of post-obstructive diuresis with the release of unilateral ureteric obstruction and a normal contralateral Kidney. GFR may

be preserved in the setting of distal tubular damage such that the kidney filters a normal volume, but there is limited free water reabsorption. Occasionally, a pronounced aquaporin channel defect may cause diminished free water absorption in this context (Schlossberg & Vaughan, 1984).

An experimental study by Li *et al.*, (2007) concluded that decreased Na+,K,+,2Cl− cotransporter type 2, the collapse of the inner medulla osmotic gradient, and suppressed phosphorylated renal aquaporins (AQP2) could be responsible for the impaired urine-concentrating capacity in the chronic post-obstructive phase following the release of ureteric obstruction. The rarity of this condition has been ascribed to compensatory retention of salt and water by the contralateral kidney (Eskild-Jensen *et al.*, 2007).

4.1.6 Obstruction and kidney stones

Obstruction has multiple consequences on the kidneys, ureters and the general well-being of the patient. In cases of obstruction associated with infection that can lead to sepsis, the consequences can be deleterious. Also, the pain caused by ureteric obstruction is significant and debilitating. The response of the ureter to obstruction by ureteric colic due to kidney stones is not well understood.

The majority of kidney stones pass spontaneously, but this is dependent mainly on stone size and ureteric anatomy (Worcester & Coe, 2008). However, the passage of a kidney/ureteric stone can be painful due to the colicky nature of ureteric spasm which has a significant impact on the patient's quality of life, work responsibilities and predisposes them to ureteric obstruction and possible sepsis. In cases of recurrent kidney stones, the condition becomes more debilitating for the patients. Hence, multiple medications have been advocated to enhance the process of stone passage. Ureteric stones can obstruct urine transport which

may cause some dynamic changes in ureteric contractility that are modulated by vasoactive substances (Paolo Santicioli & Maggi, 1998). Ureteric obstruction was tested in experimental animal models that have shown an increase in renal blood flow (RBF) for an approximate period of 90 minutes which occurs immediately after obstruction, this phenomenon then decreases gradually after this initial response. At the level of the collecting system, there is a rise in pressure in the initial five hours; then the pressure starts to decrease (Moody, Vaughn & Gillenwater, 1975).

The mediation of the increase in the renal blood flow is mainly through the action of nitric oxide and eicosanoids such as prostaglandin E2 and I2. (Allen et al. 1978; Morrison et al. 1977; Chevalier et al. 1992). Prostaglandins are produced in the kidney and play an essential role in triggering the inflammatory response, contributing to the inception and progression of kidney diseases (Li *et al.*, 2018).

The action of angiotensin II and endothelin then decreases the renal blood flow (Pimentel *et al.*, 1993). Due to the obstruction, there is an increase in intraluminal pressure proximal to the point of obstruction with an increase in ureteric diameter. At this point of ureteric obstruction, there is a transient increase in the frequency and amplitude of smooth muscle contraction, which diminishes over a period. The urine flow is then dependant on the hydrostatic pressure of the urine made by the kidney (Rose & Gillenwater, 1973, 1978).

During obstruction, there is a risk of infection which may cause an inflammatory response, further hindering the flow of urine. The effect of the stone on the intraluminal aspect of the ureter is also a factor that will impact urine flow as the ureteric mucosa can become inflamed and oedematous which will affect the ureteric compliance and the intraluminal diameter which will further inhibit the passage of the stone (Holmlund & Hassler, 1965). The

inflammation and oedema affecting the ureteric mucosa may cause inflammation and ischemia that leads to ureteric stricture (Fam *et al.*, 2015).

Ureteric stones

The size and position of ureteric stones affect the passage rate, and the majority of the stones are small in size and pass spontaneously. In the prospective study by Miller and Kane, they investigated the time to spontaneous stone passage using plain radiography films. For stones <2mm wide - took 31 days to pass. 2-4mm wide - 40 days, and 4-6mm 39 days for the stones to clear from the ureter (Miller & Kane, 1999). The series by Coll et al. used non-contrast computed tomography (CT) which is the gold standard for detecting urinary tract stones; they show similar results (Coll, Varanelli & Smith, 2002).

Tchey et al. have shown similar results of patients with ureteric stones in a series of 656 ureteric stones, 86.3% (566) of patients passed the stone spontaneously. The mean time to stone passage was 6.8 days for stones less than 2mm in size, 12.6 days for stones 2 to 4mm, 14.8 days for stones 4 to 6mm and 21.8 days for stones 6 to 8mm. The passage rate of stones was 55.3% in 7 days, 73.7% in 14 days, 88.5% in 28 days, and 97.7% in 60 days after the first episode of renal colic. Ninety (90) patients (13.7%) required intervention due to recurrence of symptoms or deterioration in renal function. The size and location of the ureteric stone are the most significant factors in predicting spontaneous passage. The more proximal the stone with a size of more than 6mm, the less chance of passing the stone (Tchey *et al.*, 2011). Ueno et al. reported that 286 of 520 (53%) patients with ureteric stones were able to pass the stones spontaneously. As described earlier, size had a significant effect on the rate of stone passage. The stones that passed spontaneously had a mean size of 6.3 mm in length and 4.0 mm in width; the stones that required intervention had a mean size of 11.7 mm in length and

7.1 mm in width. They reported that larger stones, more than 8mm were unlikely to pass (Ueno *et al.*, 1977). The cohort investigated by Morse and Resnick show that ureteric calculi with smaller size and more distal in the ureter had higher chances of spontaneous passage. They calculated the stone width and position in the ureter and stratified the findings as follows:

1 mm width, middle ureter 85%, distal ureter 100%.

2 mm width, proximal ureter 100%, middle ureter 83%, distal ureter 93%.

3 mm width, proximal 42%, middle 55%, distal 69 %.

4 mm width, proximal 20%, middle 62%, distal 55%.

5 mm width, proximal 6%, middle 57%, distal 45%.

6 mm width, distal 25%.

This cohort shows that patients with smaller stones located in the middle and distal ureter have a higher chance of spontaneous stone passage (Morse & Resnick, 1991). More than 80% of stones pass spontaneously, the size and location of the stone determines the likelihood of spontaneous passage (Jendeberg *et al.*, 2017).

4.1.7 Medical expulsive therapy for stone passage

The passage of ureteric calculi may induce smooth muscle spasm that may affect the passage of stones and cause the classic symptoms of ureteric colic. There are multiple pharmacologic agents with different actions that have been used to facilitate stone passage. The ideal pharmacologic agent would have a spasmolytic action but still maintain peristalsis to aid in the passage of the stone.

Calcium channel blockers

This class of medication which inhibit the influx of extracellular calcium has been used to facilitate the passage of ureteric stones. Nifedipine, diltiazem and verapamil have been shown to inhibit ureteric contraction in humans and guinea pigs (Golenhofen & Lammel, 1972)(Forman *et al.*, 1978). The study by Hertle and Nawrath show that verapamil and nifedipine suppressed fast phasic contractions which are ureteric spasms without affecting slow tonic contractions, this suggests that calcium channel blockers can inhibit ureteric spasm without affecting peristalsis (L Hertle & Nawrath, 1984).

Alpha blockers

The autonomic nervous system modulates the contractile activity of the ureteric smooth muscles. The stimulation of α-1 adrenergic receptors enhances the contractile amplitude and frequency of the ureteric smooth muscles. α1- adrenoceptors have been identified both in animal and human ureters. Activation of α1- adrenoceptors can stimulate the PLC, IP3, DAG pathway and induce ureteric contraction. The density of α1 -adrenoceptors (α1A and α1D) in the ureteral smooth muscle is higher than other adrenoceptors (Morita *et al.*, 1994; Scofield *et al.*, 1995; Sigala *et al.*, 2005).

The rationale behind using a blocking agent that antagonises the activity of α-1 receptors may decrease the contractile activity associated with the ureteric spasm caused by the stone and subsequently facilitate stone passage.

The study by Lindsey et al. show that the implantation of stones in canine ureters induced hyperperistaltic waves of contractions that created higher pressures than in the control group. The administration of the nonselective antagonist phenoxybenzamine decreased this spasmodic episode (Lindsey *et al.*, 1979).

The investigation by Peters and Eckstein demonstrate that phentolamine which is a nonselective antagonist decreased the frequency of contraction in obstructed and partially obstructed canine ureters but without a significant effect on the amplitude of contraction, there is a significant increase in urinary flow in partially obstructed ureters (Peters & Eckstein, 1975).

Cervenakov et al. in a nonrandomized study administered 0.4 mg of Tamsulosin (a selective α-1 antagonist) daily to patients with ureteric stones n=51. The mean stone width was 4.0 mm, and the mean length was 7.6 mm. This group were compared with a similar number of patients with ureteric stones of similar dimensions, the mean width was 3.8 mm, and the mean length was 7.5 mm. The latter group received standard supportive care. In the Tamsulosin group, 41 of the 51 patients (80.4%) passed the stones spontaneously in comparison with 32 (62.8%) in the conservative management group. The time to stone passage was also shortened in the treated group (Cervenàkov *et al.*, 2002). There has been considerable debate about the use of expulsive medical therapy in the management of ureteric stones targeting the upper and lower segments of the ureter. The randomised controlled trial by Dellabella et al. demonstrate that Tamsulosin may be useful in facilitating the passage of distal ureteric stones (Dellabella et al. 2003).

4.1.8 THE SUSPEND trial

SUSPEND investigated the effectiveness of medical expulsive therapy in adults with ureteric colic in a multicentre, randomised, placebo-controlled trial.

Patients between the age of 18–65 years who were expected to undergo expectant management of ureteric colic with a single stone confirmed on plain computed tomography (CT) scan were recruited in 24 UK hospitals. A remote randomisation system randomly

assigned participants to tamsulosin 400 µg, nifedipine 30 mg, or placebo was taken daily for up to 4 weeks, using an algorithm considering stone size (</=5 mm or >5 mm), and stone location (upper, mid, or lower ureter).

The trial concluded that Tamsulosin 400 µg and nifedipine 30 mg are not effective at decreasing the need for further treatment to achieve stone clearance in 4 weeks for patients with expectantly managed ureteric colic (Pickard *et al.*, 2015).

Ye *et al.*, (2018) conducted a double-blind, placebo-controlled study of 3296 patients with distal ureteric stones, across 30 centres in China, to evaluate the efficacy and safety of tamsulosin. The study concluded that the use of tamsulosin is beneficial in distal ureteric stones as it facilitates stone passage and relieves renal colic. The subgroup analyses found that tamsulosin provides a superior expulsion rate for stones >5mm, but no effect for stones </=5mm.

The effectiveness of expulsive medical therapy is still under debate as large clinical trials have shown varying results in terms of stone passage, pain control during renal colic. Also, the reduction in the number of interventional procedures to clear the stones has both healthcare and financial implications; these mixed results may not be solely related to the type of medical therapy but due to our evolving understanding of ureteric physiology and the need to further understand the possible differences between the ureteric segments.

Side effects of medical expulsive therapy

The common side effects of calcium channel blockers are abdominal pain; dizziness; drowsiness; flushing; headache; nausea; palpitations; peripheral oedema; skin reactions; tachycardia and vomiting.

Uncommon side effects are angioedema; erectile dysfunction; gingival hyperplasia; myalgia; paraesthesia and syncope (BNF: NICE, 2019).

The common side effects of alpha-blockers are dizziness and sexual dysfunction. The uncommon side effects are asthenia; constipation; diarrhoea; headache; nausea; palpitations; postural hypotension; rhinitis; skin reactions and vomiting. The rare side effects are angioedema; Stevens-Johnson syndrome; syncope. Dry mouth; epistaxis and vision disorders do occur but with unknown frequency (BNF: NICE, 2019).

Nonsteroidal Anti-Inflammatory Drugs (NSAIDs)

Non-steroidal anti-inflammatory drugs (NSAIDs) have spasmolytic properties and commonly used in the management of ureteric colic. NSAIDs demonstrate various mechanisms that make them beneficial in kidney and ureteric stone disease with their effect on pain management and facilitation of stone passage. These benefits include the reduction in renal blood flow that decreases the pressure in the kidney collecting system. NSAIDs also relax the ureteric smooth muscle and reduce oedema caused by the stone impinging on the ureteric wall. In vitro studies demonstrate that prostaglandins increase the contractility of ureteric smooth muscle cells (Cole, Fry & Shuttleworth, 1988; Zwergel, Zwergel & Ziegler, 1991). NSAIDs inhibit cyclooxygenase (COX), which is an enzyme involved in prostaglandin synthesis from fatty acids.

Diclofenac which is a non-selective COX inhibitor and NS-398 is a selective COX-2 inhibitor which has anti-inflammatory and analgesic properties (Futaki *et al.*, 1994). Both agents diclofenac and NS-398 were shown to inhibit ureteric contractions (Mastrangelo *et al.*, 2000). Furthermore, celecoxib (a selective COX-2 inhibitor) and indomethacin (non-selective COX

inhibitor) both inhibit PG release in the ureter even in the presence of COX-2 induction (Jerde *et al.*, 2005). As a result, selective COX-2 inhibitors may be useful with fewer side effects in the management of patients with ureteral colic.

The role of NSAIDs to facilitate ureteric stone passage has been tested in randomised controlled trials. The study by Laerum et al. show that oral diclofenac did not increase the rate of stone expulsion compared to placebo. However, diclofenac significantly decreased pain and the rate of hospital admissions (Laerum *et al.*, 1995).

Kapoor et al. demonstrate similar findings with indomethacin suppositories which is a nonselective COX inhibitor; patients with ureteric stones did not have an increased stone passage rates or decreased time to the stone passage in comparison with patients receiving placebo. Furthermore, the patients receiving indomethacin did require a statistically significant lower dose of narcotic analgesia (Kapoor *et al.*, 1989).

4.2 Hypothesis

In this chapter, the effect of stones on ureteric contractility is investigated.

In chapter 2 of this thesis, the nature of contraction of the different ureteric segments were investigated and behavioural differences between the pelvicalyceal system, renal pelvis, upper ureter, middle ureter and lower ureteric segments concerning frequency and amplitude of contraction were found. To further explore this concept, we hypothesise that:

- The upper and lower ureteric segments respond differently to stone passage.

4.3 Aims

1- Develop an in vitro model of ureteric obstruction.

2- Compare the upper and lower ureteric segments basal contraction with and without obstruction.

3- Measure the effect of a cholinergic agonist on stone passage.

4.4 Methods

The methods for this chapter are explained in chapter 2.

4.5 Results:

The upper ureteric (UU) segments n=14 and lower ureteric (LU) segments n= 18 both demonstrated basal spontaneous activity.

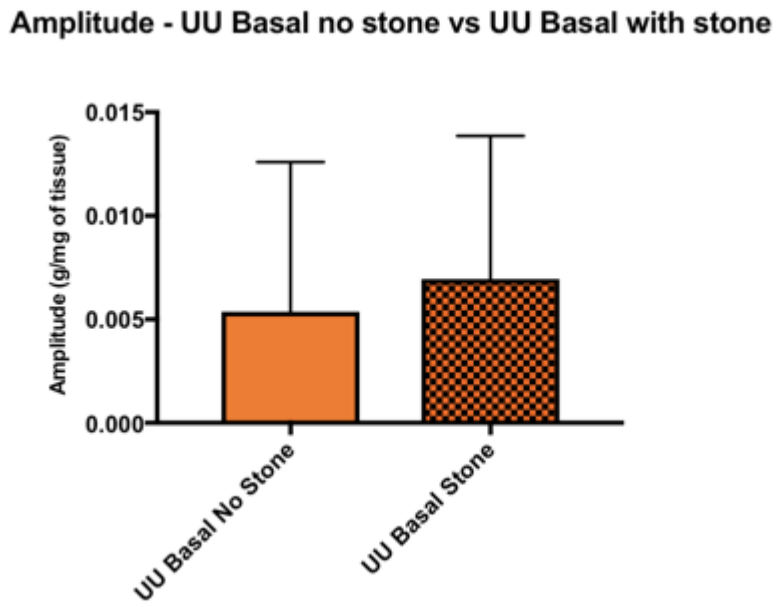
The UU and LU segments were further divided into

1- **Upper ureteric segments with a stone (UU(+)Stone)** n=7 - to investigate the effect of stone passage on the basal ureteric contractility of the upper ureteric segments and the effect of Carbachol on stone passage recording both amplitude and frequency of contraction.

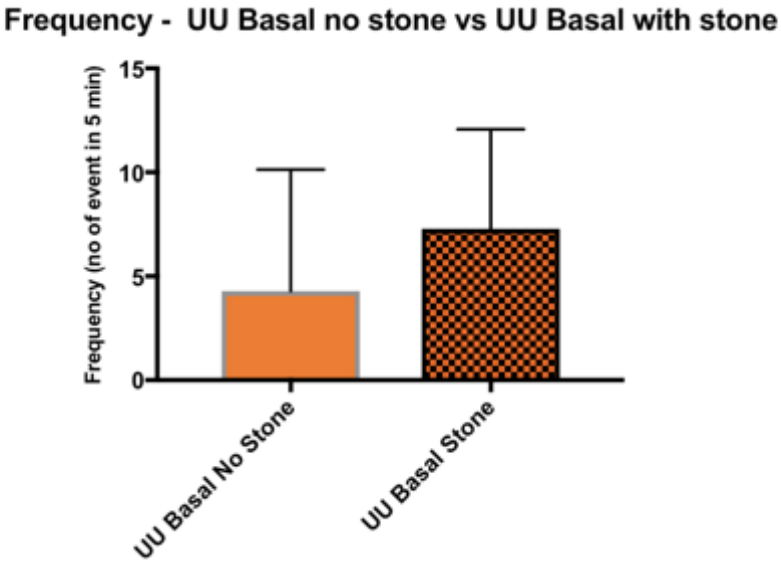
2- **Upper ureteric segments without a stone (UU(-)Stone)** n=7 - to investigate these segments as a control, recording both amplitude and frequency of basal and Carbachol-stimulated contraction.

3- **Lower ureteric segments with a stone (LU(+)stone)** n=9 - to investigate the effect of stone passage on basal ureteric contractility of the lower ureteric segments and the effect of Carbachol on stone passage recording both amplitude and frequency of contraction.

4- **Lower ureteric segments without stone (LU(-)stone)** n=9 - to investigate these segments as a control, recording both amplitude and frequency of basal and Carbachol-stimulated contraction.



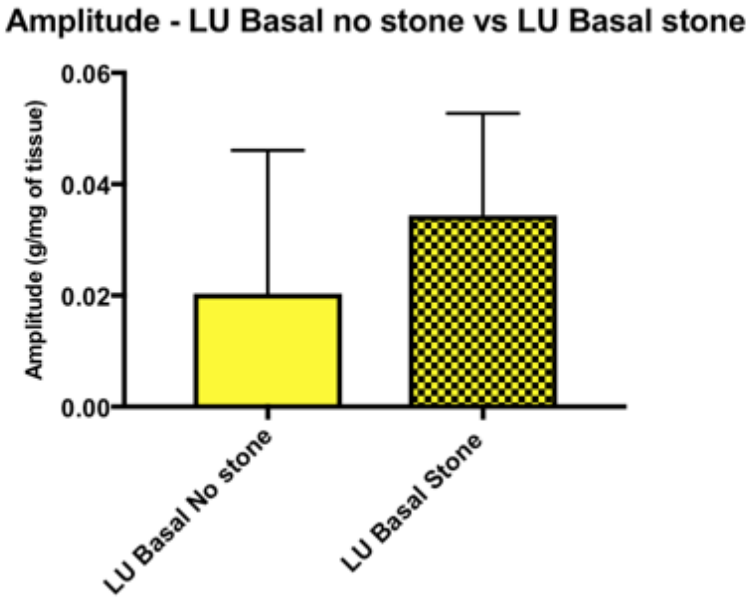
Chapter 4. Fig 1- Comparison between the amplitude of basal contraction of the UU(-)stone and UU(+)stone. There is no significant increase in amplitude of contractility in the segments with an intraluminal stone.



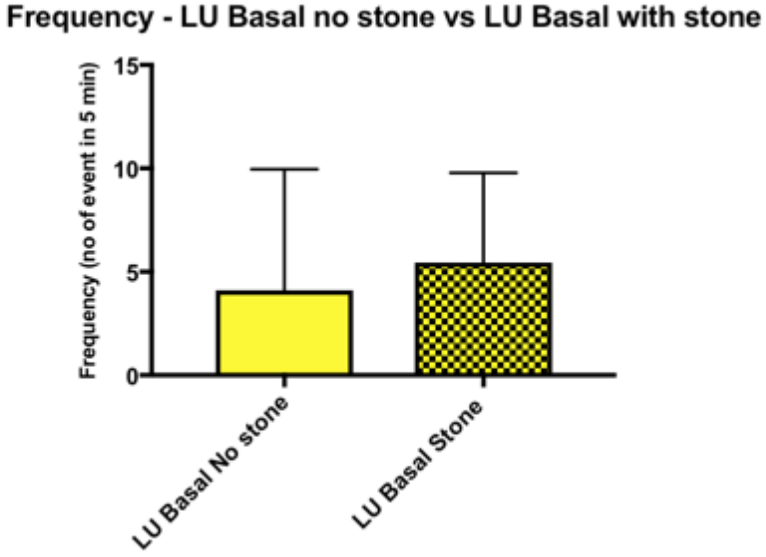
Chapter 4. Fig 2- Comparison between the frequency of basal contraction of the UU(-)stone with the frequency of UU(+)stone. There is no significant increase in contractile frequency of the upper ureteric segments with an intraluminal stone.

Fig 1 and Fig 2. The UU(-)stone were compared with UU(+)stone; this showed a slight increase in amplitude from (0.00536±0.0027 g/mgT) to (0.00695±0.0026 g/mgT). There was also

increase in contractile frequency in the UU(+)stone (7.2±1.81E/5min) in comparison with the UU(-)stone (4.3±2.21 E/5min), both amplitude and frequency of contraction were not statistically significant (P=0.6830).

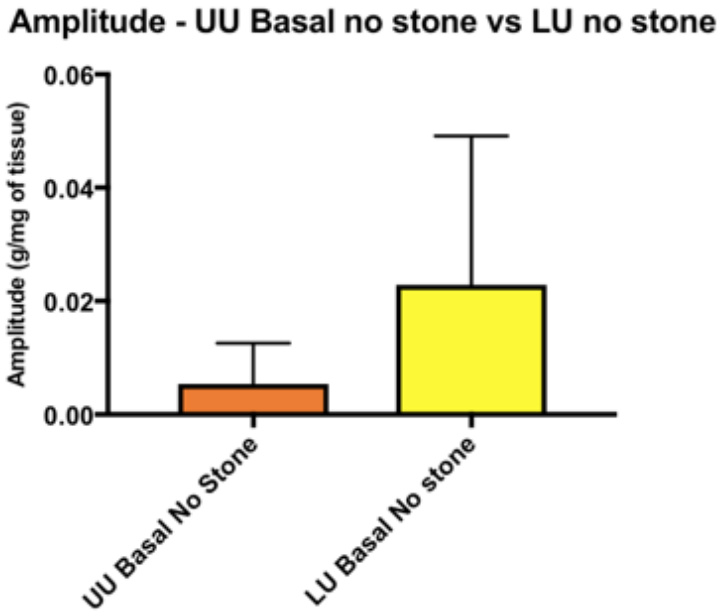


Chapter 4. Fig.3- Comparison between the amplitude of basal contraction of the LU(-)stone with the amplitude of the LU(+)stone in the lumen.

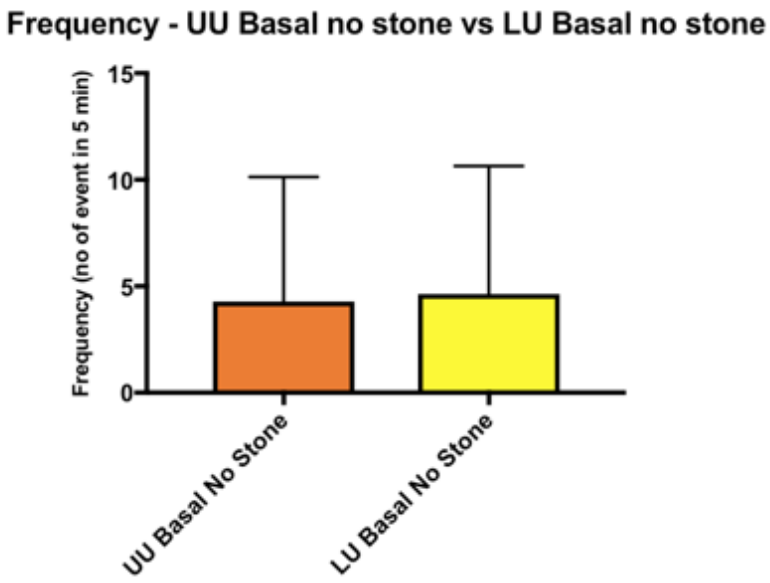


Chapter 4. Fig.4- Comparison between the frequency of basal contraction of the LU(-)stone with the frequency of the LU(+)stone.

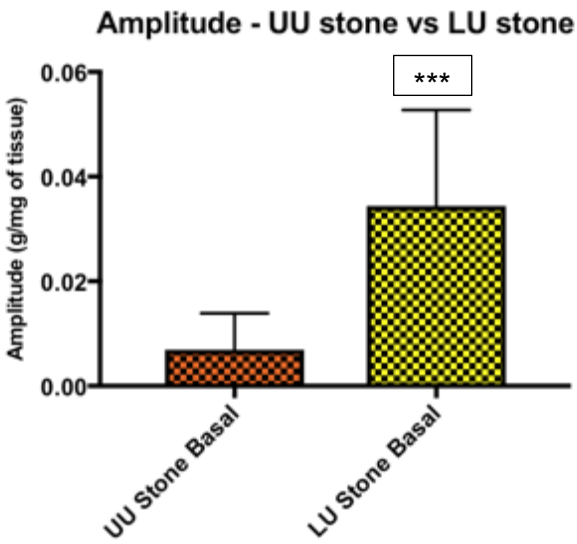
Fig 3 and Fig 4. The amplitude (0.02475 ± 0.0105 g/mgT) and frequency (5 ± 2.42 E/5min) of the lower ureteric basal contractility (LU(-)stone) was compared with the amplitude (0.04118 ± 0.0051 g/mgT) and frequency (5.57143 ± 1.67 E/5min) of the lower ureteric segment with a stone (LU(+)stone). There was no significant increase in amplitude (P=0.2017) and frequency (P=0.5531) of contraction.



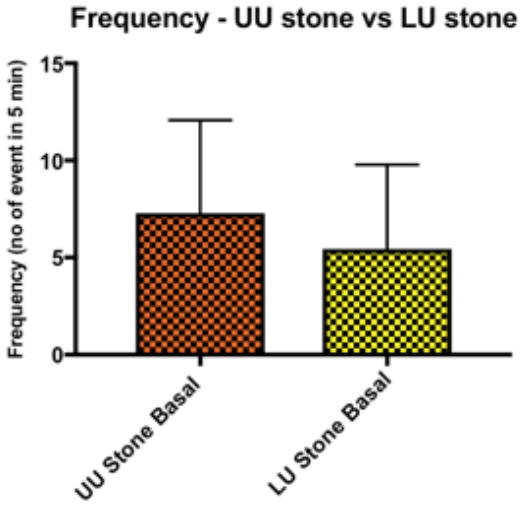
Chapter 4. Fig.5- Comparison between the amplitude of basal contraction of the UU(-)stone with the LU(-)stone.



Chapter 4. Fig.6- Comparison between the frequency of basal contraction of the UU(-)stone with the LU(-)stone. Fig 5 shows the comparison between the amplitude of contraction of the basal contractility of the upper (0.00536±0.0027 g/mgT) and lower ureteric segments (0.02287 ± 0.00929 g/mgT) without a stone and Fig 6 shows the comparison between the basal contractile frequency of upper (4.28571 ± 2.21E/5min) and lower (4.625 ± 2.13 E/5min) ureteric segments. The amplitude (P=0.1072) and frequency (P= 0.9137) of contraction did not show significant change in the lower ureteric segments.



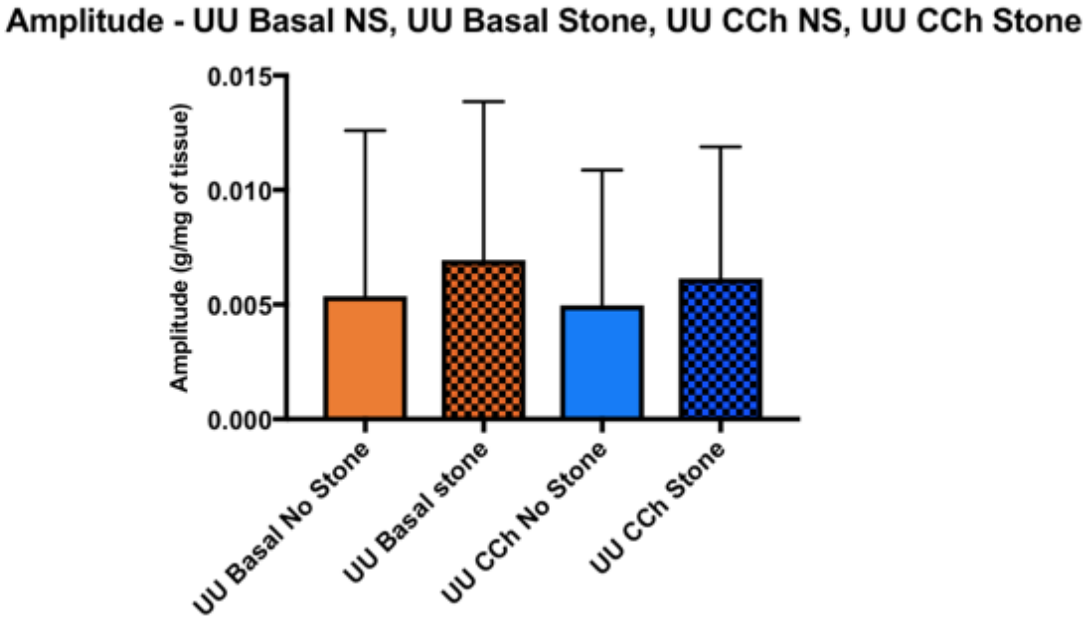
Chapter 4. Fig.7- Comparison between the amplitude of basal contraction of the UU(+)stone with the amplitude of the LU(+)stone.



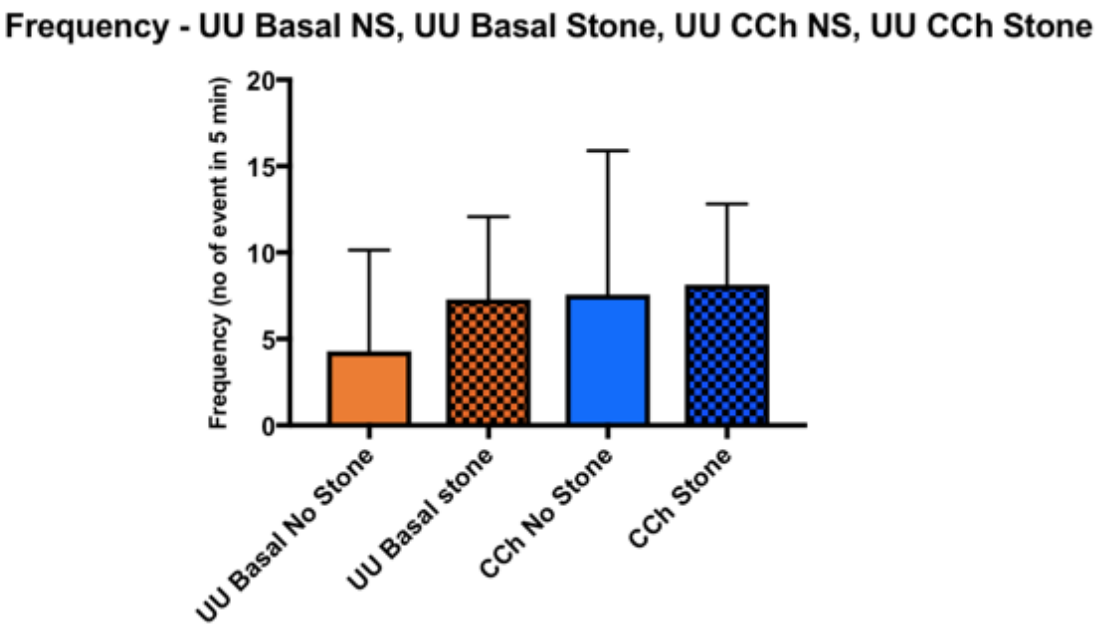
Chapter 4. Fig.8- Comparison between the frequency of basal contraction of the UU(+)stone with the frequency of the LU(+)stone.

Fig 7 shows the comparison between the upper ureteric segments (+) stone and the lower ureteric segments (+) stone. The LU segment (0.03436 ± 0.0061 g/mgT) has shown a significant increase in amplitude (P=0.0018) in comparison with the amplitude (0.00695 ± 0.0026 g/mgT) of the upper ureteric segment. Fig 8 shows the frequency of ureteric

contractility in the upper ureteric segment (7.28571 ± 1.81E/5min) in comparison with the lower ureteric segment (5.444± 1.44 E/5min), the changes were not statistically significant (P=0.4414).



Chapter 4. Fig.9 - Comparison between the amplitude of basal contraction of the UU(-)stone versus the UU(+)stone compared with the UU segments (-)stone that has been stimulated with CCh (Carbachol 10µmol) with the amplitude of the UU(+)stone that has also been stimulated with CCh (Carbachol 10µmol).



Chapter 4. Fig.10 - Comparison between the frequency of basal contractions of the UU(-)stone versus the UU(+)stone compared with the UU segments (-)stone that has been stimulated with CCh (Carbachol 10µmol); with the frequency of contraction of the UU segments (+)stone and CCh (Carbachol 10µmol).

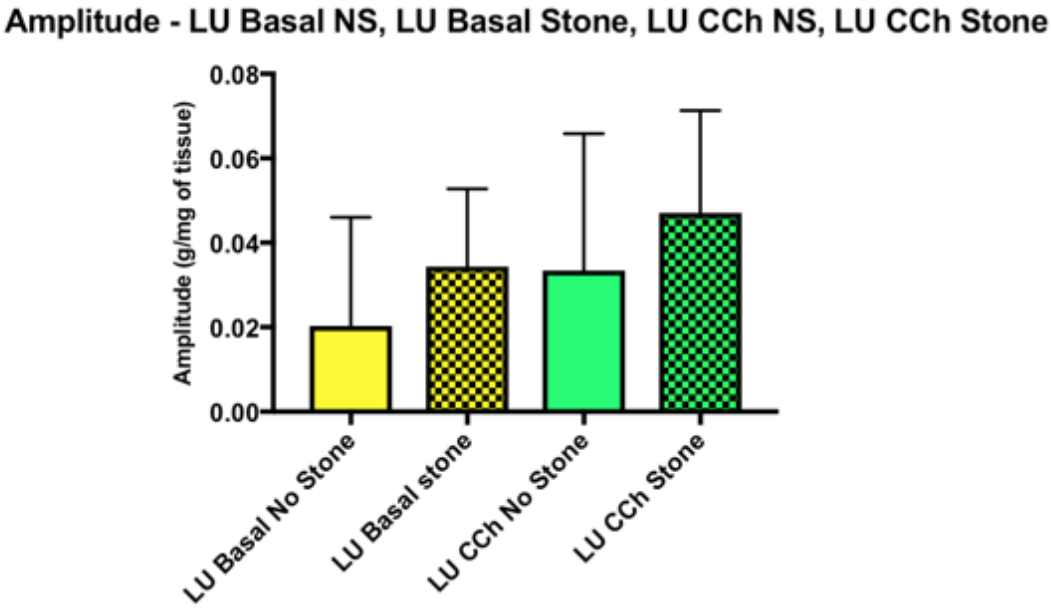
Fig 9 shows an increase in amplitude in the upper ureteric segment (+) stone (0.00695 ± 0.0026 g/mgT) in comparison with the UU basal contraction (-) stone (0.00536±0.0027g/mgT), UU CCh stimulated segment without (-) stone (0.004963654±0.0022) and the ureteric segment with (+) stone and Carbachol did also have an increase in amplitude (0.00614±0.0022 g/mgT) similar to the UU segment with a (+) stone.

Fig 10 shows the comparison between the contractile frequency of the upper ureteric segments. The ureteric stone and Carbachol each had an impact on increasing the contractile frequency of the segments they were applied to.

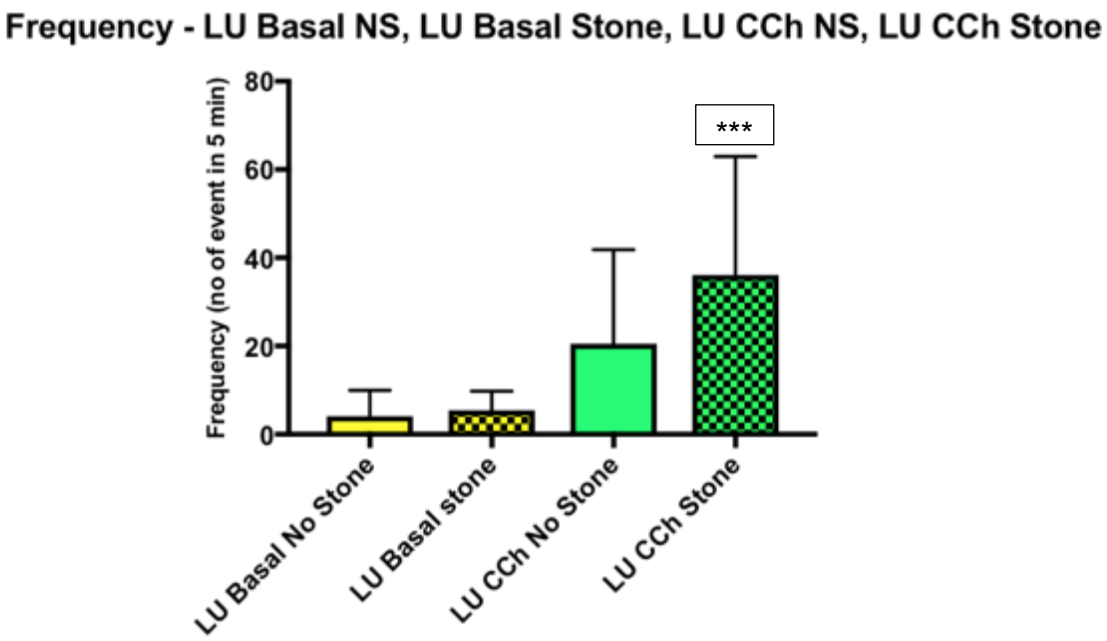
The contractile frequency of the UU segment with no (-) stone (4.29 ±2.21E/5min) has shown the lowest frequency of contraction in comparison with the basal contractility of the UU segment with a (+) stone (7.286±1.80 E/5min), UU segment with no (-) stone and stimulated

with CCh (7.5714±3.15 E/5min) and the UU segment (+) stone and stimulated with CCh (8.1429±1.7651E/5min).

Both the stone in the ureter and Carbachol did increase the contractile frequency slightly higher than the previous segments but not statistically significant. Carbachol does not seem to affect the upper ureteric segments contractile amplitude but has an effect on the contractile frequency similar to the effect of stone passage.



Chapter 4. Fig.11 – Comparison between the amplitude of basal contraction of the LU(-)stone versus the LU(+)stone and compared with LU(-)stone that has been stimulated with CCh 10µmol with the amplitude of the LU(+)stone and stimulated with CCh 10µmol.

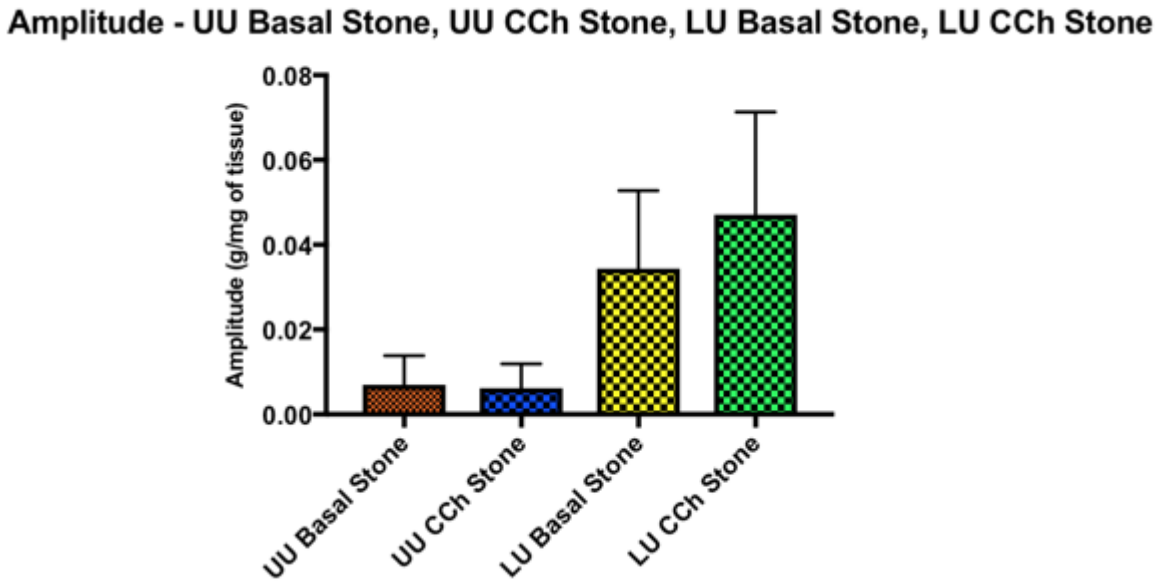


Chapter 4. Fig 12 – Comparison between the frequency of basal contraction of the LU(-)stone versus the LU(+)stone and compared with LU segment (-)stone stimulated with CCh 10µmol and LU segment (+)stone that has also been stimulated with CCh 10µmol.

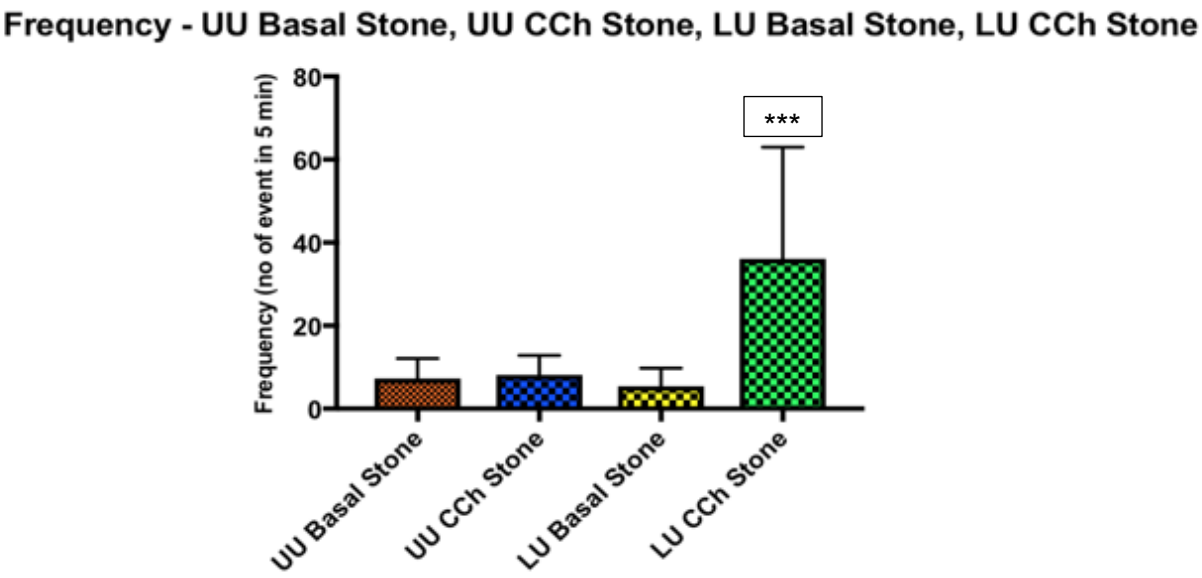
Fig 11 shows a gradual rise in amplitude with the stone having a positive effect on increasing the amplitude of contractility in the lower ureteric segments. The amplitude (0.02033±0.0086 g/mgT) of the non-stimulated LU segment without (-) a stone was the lowest among all segments. The non-stimulated LU segment with a (+) stone has shown a higher amplitude (0.03436±0.0061 g/mgT) in comparison with the previous segment but similar amplitude (0.03341±0.01081 g/mgT) to the CCh stimulated LU segment with (-) no stone. The LU segment with a (+) stone and stimulated with Carbachol demonstrated the highest amplitude (0.04708±0.0081 g/mgT) of contraction but not statistically significant (P=0.2019).

Fig 12 demonstrates that the frequency (5.44±1.44E/5min) of contraction of the non-stimulated LU segments with a (+) stone was slightly higher than the frequency (4.11±1.95

E/5min) of the non-stimulated LU segment without a (+) stone. The LU segments that were stimulated with Carbachol demonstrated a statistically significant increase in ureteric contractile frequency. The LU segment with a (+) stone and stimulated with Carbachol demonstrated a statistically significant (p=0.0013) increase in frequency (36.11±8.95 E/5min) in comparison with the non-stimulated segments with no (-) stone. There was no statistically significant difference between the LU segment stimulated with Carbachol, and the LU segment with a (+) stone stimulated with Carbachol (P=0.1895). There seems to be a synergistic effect of stones on the amplitude and frequency of contractility in the presence of Carbachol.



Chapter 4. Fig.13 – Comparison between the amplitude of basal contraction of the UU segments (+) stone versus the UU(+)stone and stimulated with CCh 10µmol, compared with the basal contractions of LU segments (+) stone and the amplitude of the LU(+)stone that has been stimulated with CCh 10µmol.



Chapter 4 .Fig.14 – Comparison between the frequency of basal contraction of the UU segments (+) stone versus UU segments with (+) stone and stimulated with CCh 10µmol, compared with basal contractions of LU segments (+) stone and the frequency of the LU segments (+) stone that has been stimulated with CCh 10µmol.

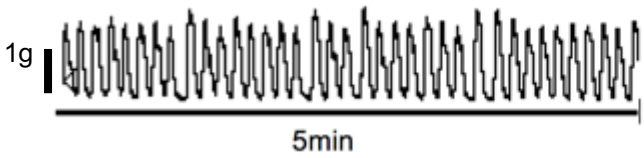
Fig 13 shows that the lower ureteric segments demonstrated statistically significant (P<0.0001) higher amplitude of contraction in both the non-stimulated (0.0344 ±0.006 g/mgT) and Carbachol-stimulated segments with a (+) stone (0.0471±0.0081 g/mgT) in comparison with both the stimulated (0.00614±0.0022 g/mgT) and non-stimulated (0.0069±0.0026 g/mgT) upper ureteric segments with (+) stone, both these segments had a similar amplitude of contraction. In the Carbachol-stimulated LU segments with a (+) stone, there was higher amplitude than the non-stimulated segment.

Fig 14 demonstrates that the frequency of contraction was similar between the basal (7.29±1.81E/5min) and Carbachol-stimulated upper ureteric segments with a (+) stone (8.14286±1.77 E/5min).

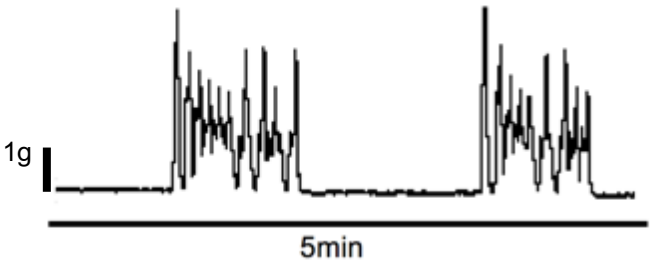
The lower ureteric segment basal contractility with (+) stone has shown slightly lower frequency (5.44±1.44E/5min) in comparison with the upper ureteric segments. The Carbachol-stimulated segment with a (+) stone of the lower ureter has shown statistically significant higher (P=0.0004) frequency (36.11±8.95 E/5min) of contraction in comparison with the other segments.

Traces of the upper and lower ureteric segments

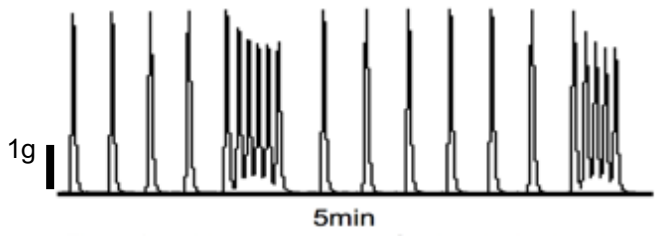
These traces show the difference between the upper and lower ureteric segments and the variety of contraction patters due to stone passage and Carbachol.



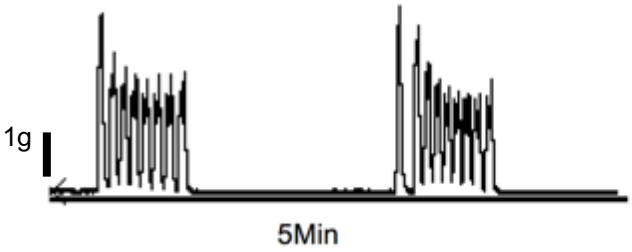
Chapter 4 – Fig 15 – Upper ureter non-stimulated basal contraction without stone.



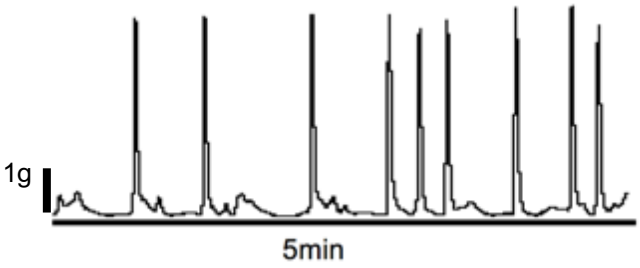
Chapter 4 – Fig 16 – Upper ureter non-stimulated basal contraction with stone.



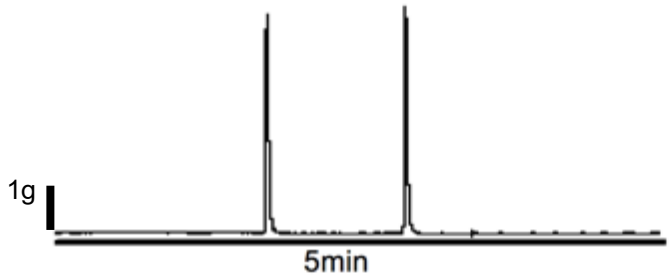
Chapter 4 – Fig 17 – Upper ureter Carbachol stimulated contraction without stone.



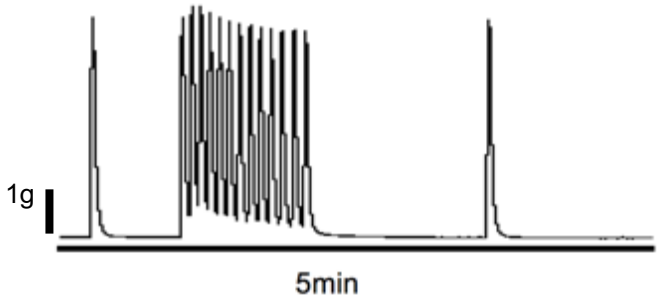
Chapter 4 – Fig 18 – Upper ureter Carbachol stimulated contraction with stone.



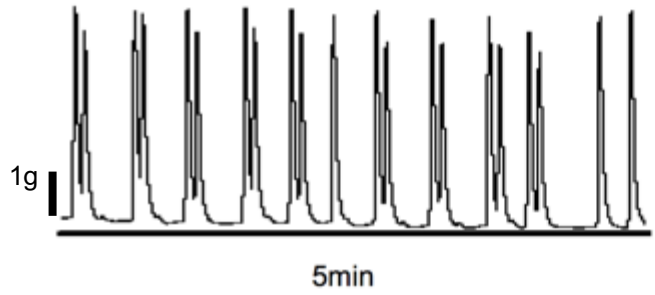
Chapter 4 – Fig 19 – Lower ureter non-stimulated basal contraction without stone.



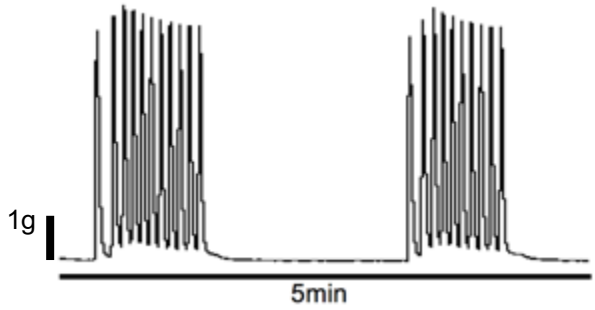
Chapter 4 – Fig 20 – Lower ureter non-stimulated basal contraction with stone.



Chapter 4 – Fig 21 – Lower ureter Carbachol stimulated contraction with stone.



Chapter 4 – Fig 22- Lower ureter Carbachol stimulated contractions without stone.



Chapter 4 – Fig 23 – Lower ureter Carbachol stimulated contractions without stone: another variety of contraction patterns.

Ureteric contractility is similar in the upper and lower segments of the ureter, yet it exhibits a difference in the pattern of contraction. This difference in contractile activity which has been measured in terms of amplitude and frequency. Also the shape of the contractile waves is related to the nature of the ureteric segment itself. Both the upper and lower ureteric segments responded to the stimulation with Carbachol and stone passage or both but the type of response is different. This concept has a clinical relevance as during renal colic; the severity of the effect of the stone passage or obstruction on a specific ureteric segment may determine the degree of pain and discomfort. The traces shown above represents a 5 min interval of ureteric contractility at basal contraction or in response to drug stimulation and/or stone passage or obstruction.

The upper ureteric segment basal contraction without a stone shows continuous and rhythmic contractions fig 15. The upper ureteric segment basal contraction with a (+) stone has shown spasmodic contractions with acontractile intervals fig 16.

The upper ureteric segment stimulated with Carbachol and without a (-) stone fig 17 shows rhythmic contractions with seemingly spasmodic intervals. Comparing the pattern of contraction between the basal upper ureteric segment and the stimulated segment denotes that Carbachol enhances this spasmodic activity during contractions. The upper ureteric segment with a (+) stone and stimulated with Carbachol, fig 18 the stone caused the upper ureter to go into spasmodic pattern of contraction with higher amplitude in comparison with basal contraction and also higher frequency in comparison with the other stimulated upper ureteric segments. The lower ureteric segment basal contraction fig 19, has shown continuous rhythmic contractions. The lower ureteric segment fig 20, with a (+) stone has shown low frequency of contraction but higher amplitude in comparison with the UU segments.

The lower ureteric segment with a (+) stone and stimulated with Carbachol fig 21, shows an increase in amplitude and frequency, with spasmodic intervals similar to the Carbachol stimulated upper ureteric segment with a (+) stone fig 18.

Fig 22 and fig 23 shows two types of contractile behaviour of the lower ureteric segments stimulated with Carbachol and without a (-) stone; this difference in behaviour was not seen in the upper ureter which could be due to the denser cholinergic innervation of the lower ureter (Prieto *et al.*, 1994; Hernández, García-Sacristán & Orensanz, 1995).

4.6 Discussion

Ureteric contractility remains interesting due to its consistency and variability at the same time. Various drugs and conditions affect ureteric contractility but also, the condition of the ureter itself affects the response to stimuli or obstruction. Nyirady et al. show that partial infravesical obstruction in foetal sheep ureters leads to diminished ureteric contractility due to the circumferential distention of the smooth muscles (Nyirády, Cuckow & Fry, 2008). The ability of the ureter to propel urine forward during peristalsis can diminish due to a period of obstruction. Biancani et al. show that there is an increase in contractile force in the rabbit ureter after two weeks of obstruction, there is no mechanical decompensation but rather the ureter undergoes changes that enhance its contractility by muscle hypertrophy and muscle fibre reorientation. Although, there are mechanical changes to overcome the obstruction; the obstructed ureter ability to generate intraluminal pressures required for urine transport decreases. This decrease in pressure results from the increase in ureteric diameter following obstruction according to Laplace's law (Biancani, Hausman & Weiss, 1982b).

Ureteric strictures and dilatation alter the electrical activity of the ureter (Shafik, 1996). In long-standing conditions like megaureters, the contractile ability of the ureters in the presence of reflux and physical or functional obstruction has a suppressive effect on the rhythmic and tonic contractions of the ureter (Mudraya *et al.*, 2004).

In the clinical setting, the ureter undergoes periods of obstruction and irritation due to stone passage. The passage of the stone depends on the stone size and the internal diameter of the ureter which may have some variability between individuals. A 2 week period of ureteric obstruction may have a significant impact on stone passage and ureteric peristaltic function. The ureter seems to have an adaptive ability to overcome obstruction and hence, judging ureteric response to expulsive medical therapy based on previous clinical trials may not show

a complete picture of how ureteric conditions and especially obstruction. The metanalysis by Hollingsworth et al. advocates the use of expulsive medical therapy, but the recent clinical trial SUSPEND reported that Tamsulosin 400 μg and nifedipine 30 mg did not show efficacy in preventing further treatment for ureteric stone patients managed conservatively (Hollingsworth *et al.*, 2006; Pickard *et al.*, 2015).

A recent trial investigated the efficacy and safety of Tamsulosin in Medical Expulsive Therapy for Distal Ureteric Stones reported that Tamsulosin had a significant effect on larger stones compared with smaller stones. The therapeutic benefit of Tamsulosin for large distal ureteral stones >5 mm ($p < 0.01$). The Tamsulosin group reported less recurrent renal colic ($p < 0.001$) and required less analgesia ($p < 0.001$) compared with placebo (Ye *et al.*, 2018).

In our current investigation, the ureter has shown behavioural variability due to pharmacological and physical stimulation. The ureteric response at different segments has been shown to be different. The relationship between ureteric obstruction, stone passage and the use of expulsive medical therapy is multifactorial and needs to take into consideration the ability of the ureter to adapt. Hence, pharmacological management of ureteric stones may target the upper and lower ureteric segments in order to facilitate stone passage but also to control the significant pain caused by the stone passage.

Chapter 5

Discussion

5.1 Introduction

The aim of this thesis was to characterise the regional spontaneous contractile properties of the ureter, and their response to a contractile agonist, and how the presence of an intraluminal obstruction would affect contractile function. The hypothesis that was tested in this thesis was that the ureter exhibits an adaptive behaviour to obstruction. The ureter should not be regarded merely as a conduit of urine but rather an active and responsive organ to pathological changes.

The ureter is a peristaltic organ with different segments contracting in a different way to fulfil its ability to conduct urine from the kidneys to the bladder. Different segments were hypothesised to have specific characteristics of function that would facilitate this process. These observations might then be used to develop novel approaches to manage more effectively ureteric stone passage and ureteric obstruction.

Several aims, using an *in vitro* model of the pig ureter, tested this hypothesis:

1. A description of the variation of spontaneous activity of the ureter in different segments
2. The effect of a contractile agonist, carbachol, on different segments of the ureter
3. The effect of obstruction on the contractile properties of the ureter.

The system begins from a high-frequency, low-amplitude contractile system on the upper part of the ureteric tract, to one of high-amplitude but low-frequency of contractions in the lower ureteric segments.

All segments of the pelvicalyceal system (PCS) and the renal pelvis demonstrated basal spontaneous activity. The PCS showed a significantly ($p<0.001$) low mean amplitude of contraction compared to the renal pelvis, middle and upper ureter. However, the mean frequency was highest of any segment compared to those from the upper ($p<0.05$), middle ($p<0.001$) and lower ($p<0.001$) ureteric segments.

Also, the renal pelvis showed a significantly ($p<0.001$) higher amplitude of contraction compared to the pelvicalyceal system ($p<0.001$) and the upper ureteric segments ($p<0.001$), similar to the middle ureter, but less than the lower ureteric segments ($p<0.05$). The contractile frequency of the renal pelvis was comparable to that of the pelvicalyceal system but significantly higher than that of the upper ($p<0.05$), middle ($p<0.001$) and lower ($p<0.001$) ureteric segments.

The amplitude of the upper ureter spontaneous activity was significantly lower than the renal pelvis, middle and lower ureteric segments ($p<0.001$) but marginally ($p<0.05$) higher than that in the pelvicalyceal system. The frequency of contraction was intermediate: it was higher than that in the middle and lower segments ($p<0.001$) but lower than that in the renal pelvis and pelvicalyceal system ($p<0.05$).

Spontaneous activity was less well-organised in middle ureter segments compared to higher regions. Inter-contraction interval was varied and of significantly lower overall frequency. Contraction amplitude was similar to the lower ureter and renal pelvis but higher than that of the upper ureter and pelvicalyceal system. In the lower ureter, characteristics were similar to that of the middle ureter, as a high-amplitude but low-frequency system.

Carbachol had no significant impact on the amplitude or frequency of contraction in the UU segments. In the MU and LU segments; Carbachol significantly increased the amplitude & frequency of contraction. Ureteric contractility is similar in the upper and lower segments of the ureter, yet it exhibits a different contractile pattern in terms of amplitude and frequency. Furthermore, the shape of the contractile waves is relevant to the nature of the ureteric segment.

The upper ureteric segments responded by slightly increased amplitude and frequency of contraction to stone passage but was not statistically significant. The lower ureteric segments responded to the stimulation with Carbachol, stone passage and both; it has shown a statistically significant increase in contractile frequency due to stone passage and carbachol. In the lower ureter there was no response to stone passage on its own and a slight increase in amplitude due to carbachol but was not statistically significant. This notion has clinical relevance as during renal colic; the severity of the effect of the stone passage or obstruction on a specific ureteric segment may determine the degree of pain and discomfort.

The upper ureteric segment basal contraction with a stone has shown spasmodic contraction pattern with acontractile intervals and higher amplitude and frequency in comparison with basal contraction in contrast with the other stimulated upper ureteric segments. The lower ureteric segment basal contraction has shown continuous rhythmic contractions. The lower ureteric segment with a stone has shown a low frequency of contraction but higher amplitude in comparison with the UU segments.

The lower ureteric segment with a stone and stimulated with Carbachol, shows an increase in amplitude and frequency, with spasmodic intervals similar to the Carbachol stimulated

upper ureteric segment with a stone. There appears to be a variation in contractile activity of the different segments of the ureter; as an integrated unit; these variations seems to facilitate the flow of urine from the kidneys to the bladder.

An important purpose to characterise the pharmacological properties of the ureter is to formulate medical expulsive therapies (MET) to facilitate kidney and ureteric stone passage and also the alleviation of renal and ureteric colic. MET is useful to facilitate the passage of ureteric stones less than 10 mm diameter and also as an adjunctive therapy with other modalities such as extracorporeal shockwave lithotripsy (ESWL) to clear 'stone-streets' (Steinstrasse). Multiple clinical trials and meta-analysis have demonstrated to some degree conflicting evidence regarding the efficacy of medical expulsive therapy, but much of the evidence is conflicting (Hollingsworth *et al.*, 2006; Pickard *et al.*, 2015).

Some of the inconsistency of these data may be due to the variability of contractile function of the ureter, or the physiological status of the patients when these studies were performed. For example, ureteric peristalsis can be affected by individual patient the state of hydration (Lang et al. 2002) and any previous obstruction or injury to the ureter. There can also be a difference between an acute presentation with a partially obstructing stone and a long-standing obstruction or ureteric stricture (Hesketh *et al.*, 2014). These factors may also impact on the peristaltic contractile wave that progresses along the ureter.

There are different types of medical expulsive therapy (MET) used for facilitating the passage of ureteric stones. Alpha-blockers, calcium channel blockers, Phosphodiesterase type 5 inhibitors and corticosteroids. Each of these medications has shown varying degrees of

success in facilitating stone passage. The SUSPEND trial which is a multicentre UK clinical trial has become a cornerstone in the argument against the use MET have shown that tamsulosin, an alpha-blocker and nifedipine, a calcium channel blocker, both did not have a significant effect on stone passage (De Coninck *et al.*, 2019). Other metanalysis and clinical trials have shown a different result, and that MET can potentially improve stone passage and also, reduce pain during colic episodes as patients require less analgesia (Ye *et al.*, 2018). The pathophysiology of renal colic is not entirely understood, but it has been proposed that increased intraluminal pressure significantly increases pain (Rose & Gillenwater, 1973).

NSAIDs are excellent analgesics in renal colic as their predominant action is through inhibition of prostaglandin synthesis. The stone passage through the ureter causes local irritation and stimulates prostaglandin production. Prostaglandins (PGF_{2a}) is involved in increasing evoked or spontaneous activity of the upper urinary tract (Zhang a Lang, 1994). Prostaglandins also increase glomerular afferent arteriolar vasodilatation and enhance vascular permeability which results in increased urine production and rise in renal pelvic pressure.

NSAIDs decrease glomerular filtration by up to 35%, which subsequently lowers renal pelvic pressure and the stimulation of stretch receptors (Perlmutter *et al.*, 1993). Furthermore, prostaglandin inhibition decreases ureteric oedema and inflammation, allowing better drainage; they also influence ureteric activity and reduce peristalsis. Moreover, NSAIDs may have a relaxing effect on ureteric smooth muscle. Indomethacin and diclofenac sodium abolished nearly the entire contractile response of ureteric muscle to electrical field stimulation (Cole et al., 1988). The role of NSAIDs and particularly diclofenac sodium is well described in the literature due to its effect in acute renal colic and the management of patient receiving shock wave lithotripsy (Cole & Fry, 1989).

In the meta-analysis by Holdgate et al., (2004), patients receiving NSAIDs were less prone to need rescue analgesia, unlikely to experience vomiting (5.8% *vs.* 19.5%) and achieved more significant reductions in pain scores. As well as relieving acute pain, oral diclofenac and oral/rectal indomethacin have both been shown to be effective in reducing the number of new colic episodes and significantly reduce further hospital admissions by 28–57% (Kapoor *et al.*, 1989; Laerum *et al.*, 1995).

5.2 The experimental Model

The experimental setup used a number of ureteric segments for these experiments. Because human ureteric tissue is scarce a pig model was used in this thesis. For *in vitro* studies, porcine tissue is frequently used as an alternative model. Several studies have reported similarities between human and porcine tissue concerning the anatomy and physiology of the upper and lower urinary tract. These include similarity in urodynamic and morphological characteristics, and neural control (Sibley, 1984; Parsons *et al.*, 2012). The whole pig ureter was used with the aim of cataloguing the behaviour of the various segments by measuring contractile amplitude and frequency. The response of the ureter to obstruction at the upper and lower segments also provided a more comprehensive view of the ureteric response to stone passage. A comparison of the effect of obstruction in the upper ureter a lower ureteric segments was useful, as urinary calculi frequently obstruct the distal part of the ureter (Ahmed *et al.*, 2015), although larger calculi will never progress along the ureter and obstruct the upper segments.

5.3 The relationship between structure and function of the ureter

The ureter is often viewed as a single unit, but the delineation of individual ureteric segments has shown that there are functional differences and each contributes to the whole function through a coordinated response.

There is a difference in the contractile behaviour between the pelvicalyceal system (PCS), renal pelvis and the upper ureter as these segments demonstrated higher contractile frequency. There was a gradual decrease in frequency at the middle and lower ureter. The PCS has a high-frequency rhythmic pattern of contraction and is consistent with the fact that this part of the upper urinary tract has an active role in the initiation of peristalsis.

The renal pelvis also demonstrated high amplitude contractions followed by a low amplitude contractions in the upper ureter and a gradual increase in amplitude in the middle and lower ureteric segments. The renal pelvis contracts strongly to overcome the resistance offered by pelviureteric junction and initiate the propulsion of urine forward to the upper ureter (Morita, Ishizuka & Tsuchida, 1981). There was, except for the renal pelvis, an inverse relationship between the frequency and amplitude of contraction as the frequency gradually decreases from the pelvicalyceal system onwards. Thus, the overall contractile work to propel urine (amplitude * frequency) remained approximately constant along the ureter. At the renal pelvis there were both large amplitude and high frequency contractions because, as noted above, this is where bulk flow of urine is initiated and so more work is required to provide this initial inertia. The significance of these regional variations has not been fully elucidated, but a number of observations may be offered.

The pelvicalyceal system showed the highest frequency, but with low amplitude contractions. The role of this segment is to transfer small volumes of urine continuously from the calyces and may have some role in initiating peristalsis.

As noted above, most contractile work is done by the renal pelvis to initiate bulk flow of urine. The upper ureter will augment the passage of the urine bolus to pass forward into the middle ureter. It offers less fluid resistance due to its relatively large diameter and so flow may be continued with small amplitude contractions.

The middle and lower ureteric segments have lower frequency and higher amplitude contractions. The lower ureteric segment has the highest amplitude of contraction and may be necessary to contract against the resistance to urine flow offered by the vesicoureteric junction. The pig ureteric dimensions and geometry seem to have an impact on the flow of urine bolus and also the passage of kidney stones (Nuss, et al. 2005). These dimensions were measured by the author of this thesis in a collaborative investigation to develop a ureteric model. The internal diameter of (n=8) pig ureters were measured. The average length of the ureters was 28.9±20 mm. The ureter was divided into three equal parts along its length; denoting the upper, middle and lower ureteric segments. The measurement was taken just distal to the renal pelvis. The measurement of the inner diameter of the ureter at the pelviureteric junction measures 6.5±1.3mm, the uppermost part of the upper ureter measures 6.0±1.3mm and tapers down to 3.0±0.6mm at the lower end of the upper ureter. The uppermost part of the middle ureter was 2.6±0.6mm, and the lower part of the middle ureter was 2.5±0.5mm. The uppermost part of the lower ureteric segment was 2.6±0.6, and the lowermost segment of the lower ureter was 2.4±0.5mm. The VUJ was slightly larger with 2.9±0.2mm (Clavica *et al.*, 2014).

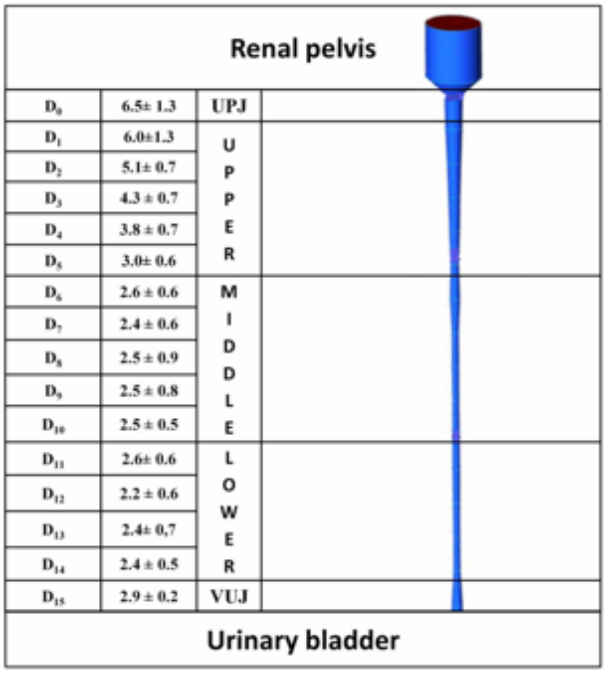


Fig 5.1- Reconstruction of pig ureter geometry. Average pig ureter internal diameters (Di , in mm; N = 8), along the longitudinal coordinate i (i = 0 at UP; i = 15 at VUJ) divided into Upper, Middle and Lower ureter (left). UPJ = ureteropelvic junction, VUJ = vesicoureteric junction. On the right; computer-aided design of the ureter. Adapted from Clavica et al (2014).

5.4 Pharmacological manipulation of the ureter

The action of carbachol on the ureter demonstrated differences between basal and agonist-induced contractile behaviour. Carbachol (10 µM) had no impact on the amplitude or frequency of contraction in the upper ureteric segments. In the middle ureter segments, carbachol (10 µM) significantly increased the amplitude and frequency of contraction. The lower ureter segments have also shown a significant response to carbachol (10 µM) with a substantial rise in contractile amplitude and frequency. Atropine (1µM) had no effect on the amplitude and frequency of contraction on the upper ureteric, middle, and lower ureteric segments.

The three segments demonstrated a difference in contractile amplitude and frequency in response to both Carbachol but not to atropine. There is a relationship between the nature of modulated ureteric behaviour concerning the change in contractile frequency and amplitude and the density of cholinergic innervation of the ureteric segments.

Muscarinic cholinergic receptors have been shown in the ureter and particularly in the distal and intravesical segments (Hernández *et al.*, 1993). It has been reported that all five types of muscarinic receptors (M1–5) are immunohistochemically identified in the human ureter, although reverse transcriptase polymerase chain reaction confirms the presence of only M2, M3 and M5 (Sakamoto *et al.,* 2006). More reliable radioligand binding studies have essentially identified M2 receptors in the porcine ureter (Hernández *et al*., 1995). The M3 receptor was found to mediate rhythmic contraction in a preparation of canine ureter, whereas relaxation was probably mediated mainly via the M4 receptor (Tomiyama *et al.*, 2003b). In the study by Tomiyama *et al.*, (2004), cholinergic receptor stimulation by the muscarinic agonist carbachol in anesthetized dogs had a suppressive effect on pressure and peristalsis in obstructed ureters in contrast to its activation of bladder smooth muscle.

Anticholinergic medications may have a relaxing effect on the smooth muscle of the urinary tract and may reduce renal colic (Schneider *et al.*, 2004). N-butylscopalamine did not reduce the opioid analgesia requirements in the management of renal colic in a randomised clinical trial (Holdgate *et al.*, 2004b). Furthermore, in the literature, there is no evidence that anticholinergics facilitate stone expulsion. Only one randomised controlled trial investigated the efficacy of tolterodine in medical expulsion therapy. Erturhan *et al.*, (2007) compared the efficacy of tamsulosin and tolterodine in 120 patients with distal ureteric stones. Patients

were divided into four groups. Group 1 patients received tamsulosin 0.4 mg/day, group 2 patients received tamsulosin 0.4 mg/day plus tolterodine 2 mg (twice a day), group 3 patients received tolterodine 2 mg (twice a day) and group 4 was the control group. The study showed that the stone expulsion rate was 73.3% for group one, 70% for group two and 46.6% and 40% for groups three and four, respectively. Tolterodine was not effective in improving the expulsion rate or reducing the expulsion time. Also, it did not contribute significantly in reducing the events of renal colic.

Cholinergic receptor agonists have not been used as expulsive medical therapy; as most medical treatments investigated were mainly to relax the ureter and hence allow the stone to pass. There is still a degree of controversy regarding the benefit of medical expulsive therapy. This thesis investigated the effect of cholinergic receptor agonist (carbachol) and whether enhanced ureteric contractility may propel the stone through the ureter more effectively. There is evidence from the data presented in this thesis that the ureter responded differently to cholinergic-receptor manipulation which demonstrates that there is potential in pursuing a more targeted approach to expulsive medical therapy.

5.5 The response to the pathological effect and the adaptation to change

The ureter demonstrates altered contractile behaviour to adapt to physiological and pathological conditions. Changes can occur after interventions that directly influence ureteric function, or secondary changes due to interventions that alter lower urinary tract function For example, after two weeks of obstruction there was an increase in contractile function of the rabbit ureter explained by muscle fibre reorientation and hypertrophy (Biancani et al., 1982). However, partial bladder outflow obstruction of foetal sheep was associated with

diminished ureteric contractility due to the circumferential distention of the smooth muscle layer (Nyirády, Cuckow & Fry, 2008).

In addition to changes of muscle function in these conditions, there are also geometrical alterations that will diminish the contractile ability of the ureter. An increase of ureter diameter can occur proximal to the site of an obstruction of the ureter; or by reflux to generate a megaureter due to an increase of lower urinary tract pressures, due say to outflow tract obstruction. These will decrease intraluminal pressure from the increase in ureteric diameter following obstruction according to Laplace's law (Biancani, Hausman & Weiss, 1982b). If these conditions are long-standing they may also have a suppressive effect on the rhythmic and tonic contractions of the ureter (Mudraya *et al.*, 2004), in part by suppression of the electrical activity of the ureter (Shafik, 1996).

In the clinical setting, the ureter undergoes periods of obstruction and irritation due to stone passage. The passage of the stone depends on the stone size and the internal diameter of the ureter which may have some variability between individuals. A two-week period of ureteric obstruction may have a significant impact on stone passage and ureteric peristaltic function. The ureter seems to have an adaptive ability to overcome obstruction and hence, judging ureteric response to expulsive medical therapy based on previous clinical trials may not show a complete picture of the ureteric response to obstruction. Clinical trials and metanalysis show a degree of contradictory evidence concerning the use of expulsive medical therapy (Hollingsworth *et al.*, 2006; Pickard *et al.*, 2015; Ye *et al.*, 2018). The ureter has shown behavioural variability due to pharmacological and physical stimulation with a degree of variable response between the different segments. The relationship between ureteric obstruction, stone passage and the use of expulsive medical therapy is multifactorial and needs to take into consideration the adaptive characteristic of the ureter.

5.6 Limitations

Although there is a similarity between the human and pig ureter, there are limitations associated with the animal model used here. The musculature of the pig ureter is similar to the human ureter, and the pig ureter propels approximately the same volume of urine. Anatomically the young pig ureter is comparable to the human ureter, but in the older pig, the calibre is proportionately larger. Cystograms and cystometrograms in the pig are very similar to those performed in humans. The peristaltic patterns and ureteric pressures are identical at all levels of the ureter in both the intact human and pig ureter. Normally the pig ureter does not show reflux as in humans (Melick & Schmidt, 1961)

The ureter was divided into segments to study it in a more detailed manner that gave considerable insight into its physiology. The investigation of the complete ureter may have given a different picture or insight due to the continuity of peristalsis, the interaction between the different parts and the integrity of the smooth muscle cells along the whole ureter.

In addition, changes to ureteric blood flow will influence the ureteric response to either medications or obstruction are not represented in an *in vitro* model.

Another limitation is the viability of the retrieved porcine tissue which limits the duration of the experiments.

5.7 Insights, recommendations for future research and practical applications

To achieve a whole picture of the nature of the upper urinary tract, various parts of its function were investigated. The upper urinary tract was divided into five segments, and the behaviour of each segment was investigated, along with the effect of cholinergic receptor modulation. The effect of obstruction was catalogued for segments of the upper and lower ureter.

The investigations of this thesis have shown that there is a functional difference in the nature of the contraction; i.e. there is a difference between the frequency and amplitude of contraction between different segments. There is also a difference between the upper and the lower ureteric segments in responding to obstruction and stone passage. The change in ureteric structure due to obstruction can alter the contractile behaviour (Nyirády, Cuckow & Fry, 2008).

Other conditions like pregnancy pose physiological and mechanical changes on the ureter due to the pressure of the uterus on the ureter and the effect of progesterone (Hickling, Sun & Wu, 2015). The functional nature of the ureter can be understood regarding a general behaviour and a changing specific and adaptable behaviour. The general behaviour is peristalsis, and the specific set of behaviours is the response of each part of the upper urinary tract to change; the adaptability to pathological conditions and the continuous drive of the general and specific behaviours towards the continuity of a single focused function which is peristalsis. Therefore a methodological approach has been adopted to develop a conceptual understanding to delineate the observations and insights gained from the investigations for this thesis.

- Medical expulsive therapy (MET) is a useful management strategy to remove ureteric stones and manage renal colic. Further investigations to catalogue the effect of MET medications on the different segments of the ureter would be useful as a step towards developing clinical trials that would test combination therapy of drugs to target the upper and lower ureteric segments either separately or simultaneously.
- Carbachol which is a cholinergic antagonist increased ureteric amplitude and frequency of contraction significantly in the middle and lower ureteric segments and

also in the presence of a stone. Bethanechol is a muscarinic agonist that has been used for the management of urinary retention and may have a similar effect to Carbachol on the ureter. Although, theoretically it may increase ureteric contractility and hence colic, combination of this drug with nonsteroidal anti-inflammatory drugs may facilitate stone passage. This may be further studied *in vitro* and also in a randomised double-blind placebo controlled clinical trial.

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